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PARP INHIBITORS, PHARMACEUTICAL COMPOSITIONS
COMPRISING SAME, AND METHODS OF USING SAME

5 BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to inhibitors of the nucleic enzyme poly(adenosine 5'-diphospho-ribose) polymerase ["poly(ADP-ribose) polymerase" or "PARP", which is also
10 sometimes called "PARS" for poly(ADP-ribose) synthetase]. More particularly, the invention relates to the use of PARP inhibitors to prevent and/or treat tissue damage resulting from cell damage or death due to necrosis or apoptosis; neural tissue damage resulting from ischemia and reperfusion injury;
15 neurological disorders and neurodegenerative diseases; to prevent or treat vascular stroke; to treat or prevent cardiovascular disorders; to treat other conditions and/or disorders such as age-related macular degeneration, AIDS and other immune senescence diseases, arthritis, atherosclerosis, cachexia, cancer, degenerative diseases of skeletal muscle
20 involving replicative senescence, diabetes, head trauma, immune senescence, inflammatory bowel disorders (such as colitis and Crohn's disease), muscular dystrophy, osteoarthritis, osteoporosis, chronic and acute pain (such as neuropathic
25 pain), renal failure, retinal ischemia, septic shock (such as endotoxic shock), and skin aging; to extend the lifespan and proliferative capacity of cells; to alter gene expression of senescent cells; or to radiosensitize hypoxic tumor cells.

30 2. Description of the Prior Art

Poly(ADP-ribose) polymerase ("PARP") is an enzyme located in the nuclei of cells of various organs, including muscle, heart and brain cells. PARP plays a physiological role in the repair of strand breaks in DNA. Once activated by damaged DNA
35 fragments, PARP catalyzes the attachment of up to 100 ADP-ribose units to a variety of nuclear proteins, including histones and PARP itself. While the exact range of functions of PARP has not been fully established, this enzyme is thought to play a role in enhancing DNA repair.

40 During major cellular stresses, however, the extensive

activation of PARP can rapidly lead to cell damage or death through depletion of energy stores. Four molecules of ATP are consumed for every molecule of NAD (the source of ADP-ribose) regenerated. Thus, NAD, the substrate of PARP, is depleted by massive PARP activation and, in the efforts to re-synthesize NAD, ATP may also be depleted.

It has been reported that PARP activation plays a key role in both NMDA- and NO-induced neurotoxicity, as shown by the use of PARP inhibitors to prevent such toxicity in cortical cultures in proportion to their potencies as inhibitors of this enzyme (Zhang et al., "Nitric Oxide Activation of Poly(ADP-Ribose) Synthetase in Neurotoxicity", *Science*, 263:687-89 (1994)); and in hippocampal slices (Wallis et al., "Neuroprotection Against Nitric Oxide Injury with Inhibitors of ADP-Ribosylation", *NeuroReport*, 5:3, 245-48 (1993)). The potential role of PARP inhibitors in treating neurodegenerative diseases and head trauma has thus been known. Research, however, continues to pinpoint the exact mechanisms of their salutary effect in cerebral ischemia, (Endres et al., "Ischemic Brain Injury is Mediated by the Activation of Poly(ADP-Ribose) Polymerase", *J. Cereb. Blood Flow Metabol.*, 17:1143-51 (1997)) and in traumatic brain injury (Wallis et al., "Traumatic Neuroprotection with Inhibitors of Nitric Oxide and ADP-Ribosylation", *Brain Res.*, 710:169-77 (1996)).

It has been demonstrated that single injections of PARP inhibitors have reduced the infarct size caused by ischemia and reperfusion of the heart or skeletal muscle in rabbits. In these studies, a single injection of the PARP inhibitor, 3-amino-benzamide (10 mg/kg), either one minute before occlusion or one minute before reperfusion, caused similar reductions in infarct size in the heart (32-42%). Another PARP inhibitor, 1,5-dihydroxyisoquinoline (1 mg/kg), reduced infarct size by a comparable degree (38-48%). Thiemermann et al., "Inhibition of the Activity of Poly(ADP Ribose) Synthetase Reduces Ischemia-Reperfusion Injury in the Heart and Skeletal Muscle", *Proc. Natl. Acad. Sci. USA*, 94:679-83 (1997). This finding has suggested that PARP inhibitors might be able to salvage previously ischemic heart or skeletal muscle tissue.

PARP activation has also been shown to provide an index of damage following neurotoxic insults by glutamate (via NMDA receptor stimulation), reactive oxygen intermediates, amyloid β -protein, n-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its active metabolite N-methyl-4-phenylpyridine (MPP⁺), which participate in pathological conditions such as stroke, Alzheimer's disease and Parkinson's disease. Zhang et al., "Poly(ADP-Ribose) Synthetase Activation: An Early Indicator of Neurotoxic DNA Damage", *J. Neurochem.*, 65:3, 1411-14 (1995).

Other studies have continued to explore the role of PARP activation in cerebellar granule cells in vitro and in MPTP neurotoxicity. Cosi et al., "Poly(ADP-Ribose) Polymerase (PARP) Revisited. A New Role for an Old Enzyme: PARP Involvement in Neurodegeneration and PARP Inhibitors as Possible Neuroprotective Agents", *Ann. N. Y. Acad. Sci.*, 825:366-79 (1997); and Cosi et al., "Poly(ADP-Ribose) Polymerase Inhibitors Protect Against MPTP-induced Depletions of Striatal Dopamine and Cortical Noradrenaline in C57B1/6 Mice", *Brain Res.*, 729:264-69 (1996).

Neural damage following stroke and other neurodegenerative processes is thought to result from a massive release of the excitatory neurotransmitter glutamate, which acts upon the N-methyl-D-aspartate (NMDA) receptors and other subtype receptors. Glutamate serves as the predominate excitatory neurotransmitter in the central nervous system (CNS). Neurons release glutamate in great quantities when they are deprived of oxygen, as may occur during an ischemic brain insult such as a stroke or heart attack. This excess release of glutamate in turn causes over-stimulation (excitotoxicity) of N-methyl-D-aspartate (NMDA), AMPA, Kainate and MGR receptors. When glutamate binds to these receptors, ion channels in the receptors open, permitting flows of ions across their cell membranes, e.g., Ca^{2+} and Na^{+} into the cells and K^{+} out of the cells. These flows of ions, especially the influx of Ca^{2+} , cause overstimulation of the neurons. The over-stimulated neurons secrete more glutamate, creating a feedback loop or domino effect which ultimately results in cell damage or death via the production of proteases, lipases and free radicals.

Excessive activation of glutamate receptors has been implicated in various neurological diseases and conditions including epilepsy, stroke, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's disease, 5 schizophrenia, chronic pain, ischemia and neuronal loss following hypoxia, hypoglycemia, ischemia, trauma, and nervous insult. Recent studies have also advanced a glutamatergic basis for compulsive disorders, particularly drug dependence. Evidence includes findings in many animal species, as well as, 10 in cerebral cortical cultures treated with glutamate or NMDA, that glutamate receptor antagonists block neural damage following vascular stroke. Dawson et al., "Protection of the Brain from Ischemia", *Cerebrovascular Disease*, 319-25 (H. Hunt Batjer ed., 1997). Attempts to prevent excitotoxicity by 15 blocking NMDA, AMPA, Kainate and MGR receptors have proven difficult because each receptor has multiple sites to which glutamate may bind. Many of the compositions that are effective in blocking the receptors are also toxic to animals. As such, there is no known effective treatment for glutamate 20 abnormalities.

The stimulation of NMDA receptors, in turn, activates the enzyme neuronal nitric oxide synthase (NNOS), which causes the formation of nitric oxide (NO), which more directly mediates neurotoxicity. Protection against NMDA neurotoxicity has 25 occurred following treatment with NOS inhibitors. See Dawson et al., "Nitric Oxide Mediates Glutamate Neurotoxicity in Primary Cortical Cultures", *Proc. Natl. Acad. Sci. USA*, 88:6368-71 (1991); and Dawson et al., "Mechanisms of Nitric Oxide-mediated Neurotoxicity in Primary Brain Cultures", *J. 30 Neurosci.*, 13:6, 2651-61 (1993). Protection against NMDA neurotoxicity can also occur in cortical cultures from mice with targeted disruption of NNOS. See Dawson et al., "Resistance to Neurotoxicity in Cortical Cultures from Neuronal Nitric Oxide Synthase-Deficient Mice", *J. Neurosci.*, 16:8, 35 2479-87 (1996).

It is known that neural damage following vascular stroke is markedly diminished in animals treated with NOS inhibitors or in mice with NNOS gene disruption. Iadecola, "Bright and

Dark Sides of Nitric Oxide in Ischemic Brain Injury", *Trends Neurosci.*, 20:3, 132-39 (1997); and Huang et al., "Effects of Cerebral Ischemia in Mice Deficient in Neuronal Nitric Oxide Synthase", *Science*, 265:1883-85 (1994). See also, Beckman et al., "Pathological Implications of Nitric Oxide, Superoxide and Peroxynitrite Formation", *Biochem. Soc. Trans.*, 21:330-34 (1993). Either NO or peroxynitrite can cause DNA damage, which activates PARP. Further support for this is provided in Szabó et al., "DNA Strand Breakage, Activation of Poly(ADP-Ribose) Synthetase, and Cellular Energy Depletion are Involved in the Cytotoxicity in Macrophages and Smooth Muscle Cells Exposed to Peroxynitrite", *Proc. Natl. Acad. Sci. USA*, 93:1753-58 (1996).

Zhang et al., U.S. Patent No. 5,587,384 issued December 24, 1996, discusses the use of certain PARP inhibitors, such as benzamide and 1,5-dihydroxy-isoquinoline, to prevent NMDA-mediated neurotoxicity and, thus, treat stroke, Alzheimer's disease, Parkinson's disease and Huntington's disease. However, it is has now been discovered that Zhang et al. may have been in error in classifying neurotoxicity as NMDA-mediated neurotoxicity. Rather, it may have been more appropriate to classify the in vivo neurotoxicity present as glutamate neurotoxicity. See Zhang et al. "Nitric Oxide Activation of Poly(ADP-Ribose) Synthetase in Neurotoxicity", *Science*, 263:687-89 (1994). See also, Cosi et al., Poly(ADP-Ribose) Polymerase Inhibitors Protect Against MPTP-induced Depletions of Striatal Dopamine and Cortical Noradrenaline in C57B1/6 Mice", *Brain Res.*, 729:264-69 (1996).

It is also known that PARP inhibitors affect DNA repair generally. Cristovao et al., "Effect of a Poly(ADP-Ribose) Polymerase Inhibitor on DNA Breakage and Cytotoxicity Induced by Hydrogen Peroxide and γ -Radiation," *Terato., Carcino., and Muta.*, 16:219-27 (1996), discusses the effect of hydrogen peroxide and γ -radiation on DNA strand breaks in the presence of and in the absence of 3-aminobenzamide, a potent inhibitor of PARP. Cristovao et al. observed a PARP-dependent recovery of DNA strand breaks in leukocytes treated with hydrogen peroxide.

PARP inhibitors have been reported to be effective in

radiosensitizing hypoxic tumor cells and effective in preventing tumor cells from recovering from potentially lethal damage of DNA after radiation therapy, presumably by their ability to prevent DNA repair. See U.S. Patent Nos. 5,032,617; 5 5,215,738; and 5,041,653.

Evidence also exists that PARP inhibitors are useful for treating inflammatory bowel disorders. Salzman et al., "Role of Peroxynitrite and Poly(ADP-Ribose)Synthase Activation Experimental Colitis," *Japanese J. Pharm.*, 75, Supp. I:15 10 (1997), discusses the ability of PARP inhibitors to prevent or treat colitis. Colitis was induced in rats by intraluminal administration of the hapten trinitrobenzene sulfonic acid in 50% ethanol. Treated rats received 3-aminobenzamide, a specific inhibitor of PARP activity. Inhibition of PARP 15 activity reduced the inflammatory response and restored the morphology and the energetic status of the distal colon. See also, Southan et al., "Spontaneous Rearrangement of Aminoalkylthioureas into Mercaptoalkylguanidines, a Novel Class of Nitric Oxide Synthase Inhibitors with Selectivity 20 Towards the Inducible Isoform", *Br. J. Pharm.*, 117:619-32 (1996); and Szabó et al., "Mercaptoethylguanidine and Guanidine Inhibitors of Nitric Oxide Synthase React with Peroxynitrite and Protect Against Peroxynitrite-induced Oxidative Damage", *J. Biol. Chem.*, 272:9030-36 (1997).

Evidence also exists that PARP inhibitors are useful for treating arthritis. Szabó et al., "Protective Effects of an Inhibitor of Poly(ADP-Ribose)Synthetase in Collagen-Induced Arthritis," *Japanese J. Pharm.*, 75, Supp. I:102 (1997), discusses the ability of PARP inhibitors to prevent or treat 30 collagen-induced arthritis. See also Szabó et al., "DNA Strand Breakage, Activation of Poly(ADP-Ribose)Synthetase, and Cellular Energy Depletion are Involved in the Cytotoxicity in Macrophages and Smooth Muscle Cells Exposed to Peroxynitrite," *Proc. Natl. Acad. Sci. USA*, 93:1753-58 (March 1996); Bauer et 35 al., "Modification of Growth Related Enzymatic Pathways and Apparent Loss of Tumorigenicity of a ras-transformed Bovine Endothelial Cell Line by Treatment with 5-Iodo-6-amino-1,2-benzopyrone (INH₂BP)", *Intl. J. Oncol.*, 8:239-52 (1996); and

Hughes et al., "Induction of T Helper Cell Hyporesponsiveness in an Experimental Model of Autoimmunity by Using Nonmitogenic Anti-CD3 Monoclonal Antibody", *J. Immuno.*, 153:3319-25 (1994).

Further, PARP inhibitors appear to be useful for treating
5 diabetes. Heller et al., "Inactivation of the Poly(ADP-Ribose) Polymerase Gene Affects Oxygen Radical and Nitric Oxide Toxicity in Islet Cells," *J. Biol. Chem.*, 270:19, 11176-80 (May 1995), discusses the tendency of PARP to deplete cellular NAD⁺ and induce the death of insulin-producing islet cells. Heller
10 et al. used cells from mice with inactivated PARP genes and found that these mutant cells did not show NAD⁺ depletion after exposure to DNA-damaging radicals. The mutant cells were also found to be more resistant to the toxicity of NO.

Further still, PARP inhibitors have been shown to be
15 useful for treating endotoxic shock or septic shock. Zingarelli et al., "Protective Effects of Nicotinamide Against Nitric Oxide-Mediated Delayed Vascular Failure in Endotoxic Shock: Potential Involvement of PolyADP Ribosyl Synthetase," *Shock*, 5:258-64 (1996), suggests that inhibition of the DNA
20 repair cycle triggered by poly(ADP ribose) synthetase has protective effects against vascular failure in endotoxic shock. Zingarelli et al. found that nicotinamide protects against delayed, NO-mediated vascular failure in endotoxic shock. Zingarelli et al. also found that the actions of nicotinamide
25 may be related to inhibition of the NO-mediated activation of the energy-consuming DNA repair cycle, triggered by poly(ADP ribose) synthetase. See also, Cuzzocrea, "Role of Peroxynitrite and Activation of Poly(ADP-Ribose) Synthetase in the Vascular Failure Induced by Zymosan-activated Plasma,"
30 *Brit. J. Pharm.*, 122:493-503 (1997).

Yet another known use for PARP inhibitors is treating cancer. Suto et al., "Dihydroisoquinolinones: The Design and Synthesis of a New Series of Potent Inhibitors of Poly(ADP-Ribose) Polymerase", *Anticancer Drug Des.*, 7:107-17 (1991),
35 discloses processes for synthesizing a number of different PARP inhibitors. In addition, Suto et al., U.S. Patent No. 5,177,075, discusses several isoquinolines used for enhancing the lethal effects of ionizing radiation or chemotherapeutic

agents on tumor cells. Weltin et al., "Effect of 6(5H)-Phenanthridinone, an Inhibitor of Poly(ADP-ribose) Polymerase, on Cultured Tumor Cells", *Oncol. Res.*, 6:9, 399-403 (1994), discusses the inhibition of PARP activity, reduced proliferation of tumor cells, and a marked synergistic effect when tumor cells are co-treated with an alkylating drug.

Still another use for PARP inhibitors is the treatment of peripheral nerve injuries, and the resultant pathological pain syndrome known as neuropathic pain, such as that induced by chronic constriction injury (CCI) of the common sciatic nerve and in which transsynaptic alteration of spinal cord dorsal horn characterized by hyperchromatosis of cytoplasm and nucleoplasm (so-called "dark" neurons) occurs. See Mao et al., *Pain*, 72:355-366 (1997).

PARP inhibitors have also been used to extend the lifespan and proliferative capacity of cells including treatment of diseases such as skin aging, Alzheimer's disease, atherosclerosis, osteoarthritis, osteoporosis, muscular dystrophy, degenerative diseases of skeletal muscle involving replicative senescence, age-related macular degeneration, immune senescence, AIDS, and other immune senescence diseases; and to alter gene expression of senescent cells. See WO 98/27975.

Large numbers of known PARP inhibitors have been described in Banasik et al., "Specific Inhibitors of Poly(ADP-Ribose) Synthetase and Mono(ADP-Ribosyl)-Transferase", *J. Biol. Chem.*, 267:3, 1569-75 (1992), and in Banasik et al., "Inhibitors and Activators of ADP-Ribosylation Reactions", *Molec. Cell. Biochem.*, 138:185-97 (1994).

However, the approach of using these PARP inhibitors in the ways discussed above has been limited in effect. For example, side effects have been observed with some of the best-known PARP inhibitors, as discussed in Milam et al., "Inhibitors of Poly(Adenosine Diphosphate-Ribose) Synthesis: Effect on Other Metabolic Processes", *Science*, 223:589-91 (1984). Specifically, the PARP inhibitors 3-aminobenzamide and benzamide not only inhibited the action of PARP but also were shown to affect cell viability, glucose metabolism, and DNA

synthesis. Thus, it was concluded that the usefulness of these PARP inhibitors may be severely restricted by the difficulty of finding a dose that will inhibit the enzyme without producing additional metabolic effects.

5

SUMMARY OF THE INVENTION

The present invention relates to a compound that inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

10 Additionally, the present invention relates to a pharmaceutical composition comprising:

(i) an effective amount of a compound that inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity; and

15 (ii) a pharmaceutically acceptable carrier.

The present invention further relates to compositions and methods for treating and/or preventing tissue damage resulting from cell damage or death due to necrosis or apoptosis, and associated diseases and conditions.

20 Moreover, the present invention relates to a method of effecting a neuronal activity not mediated by NMDA toxicity in an animal, comprising administering to the animal an effective amount of a compound that inhibits PARP activity.

The present invention also relates to a method of treating
25 a cardiovascular disorder in an animal, comprising administering to the animal an effective amount of a compound that inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

The present invention also relates to a method of treating
30 organ damage due to transplantation in an animal, comprising administering to the animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

The present invention further relates to a method of
35 treating an inflammatory bowel disorder in an animal, comprising administering to the animal an effective amount of a compound that inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

Moreover, the present invention relates to a method of

treating arthritis in an animal, comprising administering to the animal an effective amount of a compound that inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

5 The present invention also relates to a method of treating diabetes in an animal, comprising administering to the animal an effective amount of a compound that inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

10 Additionally, the present invention relates to a method of treating septic shock in an animal, comprising administering to the animal an effective amount of a compound that inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

The present invention further relates to a method of 15 treating cancer in an animal, comprising administering to the animal an effective amount of a compound that inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

The present invention further relates to methods of 20 radiosensitizing hypoxic tumor cells, comprising administering an effective amount of a compound that inhibits PARP activity and radiosensitizes the tumor cells.

The present invention further relates to methods of extending the lifespan and proliferative capacity of cells 25 comprising administering an effective amount of a compound that inhibits PARP activity.

The present invention further relates to methods of altering gene expression of senescent cells, comprising administering an effective amount of a compound that inhibits 30 PARP activity.

The present invention further relates to a method of inhibiting PARP activity in an animal, comprising administering to the animal an effective amount of a compound that inhibits PARP activity and effects a neuronal activity not mediated by 35 NMDA toxicity.

Finally, the present invention relates to methods for administering an effective amount of a compound to treat tissue damage resulting from cell damage or death due to necrosis or apoptosis, neural tissue damage resulting from ischemia and

reperfusion injury, or neurological disorders and neurodegenerative diseases; to prevent or treat vascular stroke; to treat or prevent cardiovascular disorders; to treat other conditions and/or disorders such as age-related macular
5 degeneration, AIDS and other immune senescence diseases, arthritis, atherosclerosis, cachexia, cancer, degenerative diseases of skeletal muscle involving replicative senescence, diabetes, head trauma, immune senescence, inflammatory bowel disorders (such as colitis and Crohn's disease), muscular
10 dystrophy, osteoarthritis, osteoporosis, chronic and/or acute pain (such as neuropathic pain), renal failure, retinal ischemia, septic shock (such as endotoxic shock), and skin aging; to extend the lifespan and proliferative capacity of cells; to alter gene expression of senescent cells; or to
15 radiosensitize hypoxic tumor cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the distribution of the cross-sectional infarct area at representative levels along the rostrocaudal
20 axis, as measured from the interaural line in non-treated animals and in animals treated with 10 mg/kg of 3,4-dihydro-5-[4-(1-piperidinyl)-butoxyl]-1(2H)-isoquinolinone.

Figure 2 shows the effect of intraperitoneal administration of 3,4-dihydro-5-[4-(1-piperidinyl)-butoxy]-
25 1(2H)-isoquinolinone on the infarct volume.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the present invention preferably inhibit PARP activity and act to treat or prevent tissue damage
30 resulting from cell damage or death due to necrosis or apoptosis. As such, they may treat or prevent neural tissue damage resulting from cell damage or death due to necrosis or apoptosis, cerebral ischemia and reperfusion injury or neurodegenerative diseases in an animal; they may extend the
35 lifespan and proliferative capacity of cells and thus be used to treat or prevent diseases associated therewith; they may alter gene expression of senescent cells; and they may radiosensitize hypoxic tumor cells. Preferably, the compounds of the invention treat or prevent tissue damage resulting from

cell damage or death due to necrosis or apoptosis, and/or effect neuronal activity, either mediated or not mediated by NMDA toxicity. These compounds are thought to interfere with more than the glutamate neurotoxicity and NO-mediated biological pathways. Further, the compounds of the invention can treat or prevent other tissue damage related to PARP activation.

For example, the compounds of the invention can treat or prevent cardiovascular tissue damage resulting from cardiac ischemia or reperfusion injury. Reperfusion injury, for instance, occurs at the termination of cardiac bypass procedures or during cardiac arrest when the heart, once prevented from receiving blood, begins to reperfuse.

The compounds of the present invention can also be used to extend or increase the lifespan or proliferation of cells and thus to treat or prevent diseases associated therewith and induced or exacerbated by cellular senescence including skin aging, atherosclerosis, osteoarthritis, osteoporosis, muscular dystrophy, degenerative diseases of skeletal muscle involving replicative senescence, age-related macular degeneration, immune senescence, AIDS and other immune senescence diseases, and other diseases associated with cellular senescence and aging, as well as to alter the gene expression of senescent cells. These compounds can also be used to treat cancer and to radiosensitize hypoxic tumor cells to render the tumor cells more susceptible to radiation therapy and to prevent the tumor cells from recovering from potentially lethal damage of DNA after radiation therapy, presumably by their ability to prevent DNA repair. The compounds of the present invention can be used to prevent or treat vascular stroke; to treat or prevent cardiovascular disorders; to treat other conditions and/or disorders such as age-related macular degeneration, AIDS and other immune senescence diseases, arthritis, atherosclerosis, cachexia, cancer, degenerative diseases of skeletal muscle involving replicative senescence, diabetes, head trauma, immune senescence, inflammatory bowel disorders (such as colitis and Crohn's disease), muscular dystrophy, osteoarthritis, osteoporosis, chronic and/or acute pain (such as neuropathic pain), renal failure, retinal ischemia, septic shock (such as

endotoxic shock), and skin aging.

Preferably, the compounds of the invention act as PARP inhibitors to treat or prevent tissue damage resulting from cell death or damage due to necrosis or apoptosis; to treat or prevent neural tissue damage resulting from cerebral ischemia and reperfusion injury or neurodegenerative diseases in an animal; to extend and increase the lifespan and proliferative capacity of cells; to alter gene expression of senescent cells; and to radiosensitize tumor cells. These compounds are thought to interfere with more than the NMDA-neurotoxicity and NO-mediated biological pathways. Preferably, the compounds of the invention exhibit an IC_{50} for inhibiting PARP in vitro of about 100 μM or lower, more preferably, about 25 μM or lower.

"Inhibition", in the context of enzymes, refers to reversible enzyme inhibition such as competitive, uncompetitive and non-competitive inhibition. Competitive, uncompetitive and non-competitive inhibition can be distinguished by the effects of an inhibitor on the reaction kinetics of an enzyme. Competitive inhibition occurs when the inhibitor combines reversibly with the enzyme in such a way that it competes with a normal substrate for binding at the active site. The affinity between the inhibitor and the enzyme may be measured by the inhibitor constant, K_i , which is defined as:

$$K_i = \frac{[E][I]}{[EI]}$$

wherein $[E]$ is the concentration of the enzyme, $[I]$ is the concentration of the inhibitor, and $[EI]$ is the concentration of the enzyme-inhibitor complex formed by the reaction of the enzyme with the inhibitor. Unless otherwise specified, K_i as used herein refers to the affinity between the inventive compounds and PARP. " IC_{50} " is a related term used to define the concentration or amount of a compound which is required to cause a 50% inhibition of the target enzyme.

The inventors have now discovered that select compounds can inhibit PARP activity and can ameliorate neural tissue damage, including that following focal ischemia and reperfusion injury. Generally, inhibition of PARP activity spares the cell from energy loss, preventing irreversible depolarization of the

neurons and, thus, provides neuroprotection. While not wishing to be bound thereby, it is thought that PARP activation may play a common role in still other excitotoxic mechanisms, perhaps as yet undiscovered, in addition to the production of
5 free radicals and NO.

The present invention relates to compounds which inhibit PARP activity and effect a neuronal activity not mediated by NMDA toxicity. The neuronal activity may be selected from the group consisting of stimulation of damaged neurons, promotion
10 of neuronal regeneration, prevention of neurodegeneration, and treatment of a neurological disorder.

Preferably, the neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical
15 damage to the spinal cord, stroke, demyelinating disease and neurological disorder relating to neurodegeneration.

A preferred neurological disorder is stroke, and a preferred neurological disorder relating to neurodegeneration is selected from the group consisting of Alzheimer's Disease,
20 Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis.

The compound of the invention may interact with PARP by forming at least one hydrogen bond with an amino acid in PARP, potentially Ser and/or Gly. Specifically, an NH group of the
25 inventive compound may form a hydrogen bond with the O atom of Gly in PARP. The length of such bond is about 1-5 Å, preferably about 2-4 Å. Additionally, an O, S or N atom of the inventive compound may form a hydrogen bond with the H atom of Ser. The length of such bond is about 1-5 Å, preferably about
30 2-4 Å.

Preferably, the compound of the invention exhibits an IC_{50} for inhibiting PARP in vitro of about 100 μM or lower, more preferably, about 25 μM or lower.

The compounds of the present invention possess one or more
35 asymmetric center(s) and thus can be produced as mixtures (racemic and non-racemic) of stereoisomers, or as individual R- and S-stereoisomers. The individual stereoisomers may be obtained by using an optically active starting material, by resolving a racemic or non-racemic mixture of an intermediate

at some appropriate stage of synthesis, or by resolving the compounds of the invention. It is understood that the compounds of the present invention encompass individual stereoisomers as well as mixtures (racemic and non-racemic) of
5 stereoisomers.

The term "isomers" refer to compounds having the same number and kind of atoms, and hence, the same molecular weight, but differing in respect to the arrangement or configuration of the atoms. "Stereoisomers" are isomers that differ only in the
10 arrangement of atoms in space. "Enantiomers" are a pair of stereoisomers that are non-superimposable mirror images of each other.

"Diastereoisomers" are stereoisomers which are not mirror images of each other. "Racemic mixture" means a mixture
15 containing equal, or roughly equal, parts of individual enantiomers. A "non-racemic mixture" is a mixture containing unequal, or substantially unequal, parts of individual enantiomers or stereoisomers.

The compounds of the invention may be useful in a free
20 base form, in the form of pharmaceutically acceptable salts, pharmaceutically acceptable hydrates, pharmaceutically acceptable esters, pharmaceutically acceptable solvates, pharmaceutically acceptable prodrugs, pharmaceutically acceptable metabolites, and in the form of pharmaceutically
25 acceptable stereoisomers. These forms are all within the scope of the invention. In practice, the use of these forms amounts to use of the neutral compound.

"Pharmaceutically acceptable salt", "hydrate", "ester" or "solvate" refers to a salt, hydrate, ester, or solvate of the
30 inventive compounds which possesses the desired pharmacological activity and which is neither biologically nor otherwise undesirable. Organic acids can be used to produce salts, hydrates, esters, or solvates such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, p-
35 toluenesulfonate, bisulfate, sulfamate, sulfate, naphthylate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentane-propionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate heptanoate, hexanoate, 2-hydroxyethanesulfonate, lactate,

maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, tosylate and undecanoate. Inorganic acids can be used to produce salts, hydrates, esters, or solvates such as hydrochloride, hydrobromide, hydroiodide, and thiocyanate.

5 Examples of suitable base salts, hydrates, esters, or solvates include hydroxides, carbonates, and bicarbonates of ammonia, alkali metal salts such as sodium, lithium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, aluminum salts, and zinc salts.

10 Salts, hydrates, esters, or solvates may also be formed with organic bases. Organic bases suitable for the formation of pharmaceutically acceptable base addition salts, hydrates, esters, or solvates of the compounds of the present invention include those that are non-toxic and strong enough to form such
15 salts, hydrates, esters, or solvates. For purposes of illustration, the class of such organic bases may include mono-, di-, and trialkylamines, such as methylamine, dimethylamine, triethylamine and dicyclohexylamine; mono-, di- or trihydroxyalkylamines, such as mono-, di-, and triethanolamine;
20 amino acids, such as arginine and lysine; guanidine; N-methyl-glucosamine; N-methyl-glucamine; L-glutamine; N-methyl-piperazine; morpholine; ethylenediamine; N-benzyl-phenethylamine; (trihydroxy-methyl)aminoethane; and the like. See, for example, "Pharmaceutical Salts," J. Pharm. Sci.,
25 66:1, 1-19 (1977). Accordingly, basic nitrogen-containing groups can be quaternized with agents including: lower alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such
30 as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides such as benzyl and phenethyl bromides.

The acid addition salts, hydrates, esters, or solvates of the basic compounds may be prepared either by dissolving the
35 free base of a PARP inhibitor in an aqueous or an aqueous alcohol solution or other suitable solvent containing the appropriate acid or base, and isolating the salt by evaporating the solution. Alternatively, the free base of the PARP inhibitor may be reacted with an acid, as well as reacting the

PARP inhibitor having an acid group thereon with a base, such that the reactions are in an organic solvent, in which case the salt separates directly or can be obtained by concentrating the solution.

5 "Pharmaceutically acceptable prodrug" refers to a derivative of the inventive compounds which undergoes biotransformation prior to exhibiting its pharmacological effect(s). The prodrug is formulated with the objective(s) of improved chemical stability, improved patient acceptance and
10 compliance, improved bioavailability, prolonged duration of action, improved organ selectivity, improved formulation (e.g., increased hydrosolubility), and/or decreased side effects (e.g., toxicity). The prodrug can be readily prepared from the inventive compounds using methods known in the art, such as
15 those described by *Burger's Medicinal Chemistry and Drug Chemistry*, Fifth Ed., Vol. 1, pp. 172-178, 949-982 (1995). For example, the inventive compounds can be transformed into prodrugs by converting one or more of the hydroxy or carboxy groups into esters.

20 "Pharmaceutically acceptable metabolite" refers to drugs that have undergone a metabolic transformation. After entry into the body, most drugs are substrates for chemical reactions that may change their physical properties and biologic effects. These metabolic conversions, which usually affect the polarity
25 of the compound, alter the way in which drugs are distributed in and excreted from the body. However, in some cases, metabolism of a drug is required for therapeutic effect. For example, anticancer drugs of the antimetabolite class must be converted to their active forms after they have been
30 transported into a cancer cell. Since most drugs undergo metabolic transformation of some kind, the biochemical reactions that play a role in drug metabolism may be numerous and diverse. The main site of drug metabolism is the liver, although other tissues may also participate.

35 A feature characteristic of many of these transformations is that the metabolic products are more polar than the parent drugs, although a polar drug does sometimes yield a less polar product. Substances with high lipid/water partition coefficients, which pass easily across membranes, also diffuse

back readily from tubular urine through the renal tubular cells into the plasma. Thus, such substances tend to have a low renal clearance and a long persistence in the body. If a drug is metabolized to a more polar compound, one with a lower partition coefficient, its tubular reabsorption will be greatly reduced. Moreover, the specific secretory mechanisms for anions and cations in the proximal renal tubules and in the parenchymal liver cells operate upon highly polar substances.

As a specific example, phenacetin (acetophenetidin) and acetanilide are both mild analgesic and antipyretic agents, but are transformed within the body to a more polar and more effective metabolite, p-hydroxyacetanilid (acetaminophen), which is widely used today. When a dose of acetanilid is given to a person, the successive metabolites peak and decay in the plasma sequentially. During the first hour, acetanilid is the principal plasma component. In the second hour, as the acetanilid level falls, the metabolite acetaminophen concentration reaches a peak. Finally, after a few hours, the principal plasma component is a further metabolite that is inert and can be excreted from the body. Thus, the plasma concentrations of one or more metabolites, as well as the drug itself, can be pharmacologically important.

The reactions involved in drug metabolism are often classified into two groups, as shown in the Table II. Phase I (or functionalization) reactions generally consist of (1) oxidative and reductive reactions that alter and create new functional groups and (2) hydrolytic reactions that cleave esters and amides to release masked functional groups. These changes are usually in the direction of increased polarity.

Phase II reactions are conjugation reactions in which the drug, or often a metabolite of the drug, is coupled to an endogenous substrate, such as glucuronic acid, acetic acid, or sulfuric acid.

TABLE II

Phase I Reactions (functionalization reactions):

- (1) Oxidation via the hepatic microsomal P450 system:
 - Aliphatic oxidation
 - Aromatic hydroxylation
 - N-Dealkylation
 - O-Dealkylation

- 5 S-Dealkylation
Epoxidation
Oxidative deamination
Sulfoxide formation
Desulfuration
N-Oxidation and N-hydroxylation
Dehalogenation
- 10 (2) Oxidation via nonmicrosomal mechanisms:
Alcohol and aldehyde oxidation
Purine oxidation
Oxidative deamination (monoamine
oxidase and diamine oxidase)
- 15 (3) Reduction:
Azo and nitro reduction
- 20 (4) Hydrolysis:
Ester and amide hydrolysis
Peptide bond hydrolysis
Epoxide hydration

Phase II Reactions (conjugation reactions):

- 25 (1) Glucuronidation
(2) Acetylation
(3) Mercapturic acid formation
30 (4) Sulfate conjugation
(5) N-, O-, and S-methylation
35 (6) Trans-sulfuration

Synthesis of Compounds

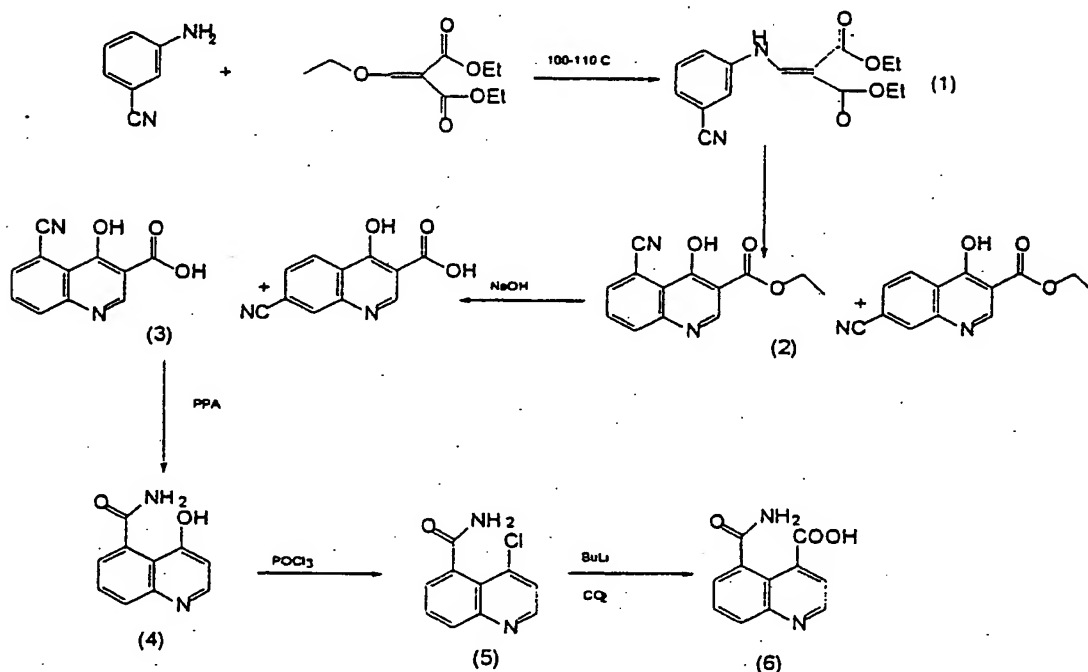
Many PARP inhibitors can be synthesized by known methods
40 from starting materials that are known, are themselves
commercially available, or may be prepared by methods used to
prepare corresponding compounds in the literature. See, for
example, Suto et al., "Dihydroisoquinolinones: The Design and
Synthesis of a New Series of Potent Inhibitors of Poly(ADP-
45 ribose) Polymerase", *Anticancer Drug Des.*, 6:107-17 (1991),
which discloses processes for synthesizing a number of
different PARP inhibitors.

Following are methods of synthesis for variously
substituted multicyclic, nitrogen-containing PARP inhibitors
50 that effect a neuronal activity not mediated by NMDA toxicity,

including carboxamide compounds, thioalkyl compounds, alkoxy-substituted compounds, amino-substituted compounds, oxo-substituted compounds, and select tricyclic compounds.

5 Synthesis of Carboxamide PARP Inhibitors

A procedure for preparation of an exemplary carboxamide PARP inhibitor, 5-carbamoylquinoline-4-carboxylic acid, is described below:



10 A mixture of m-cyanoaniline (1.0 g, 8.46 mmol) and diethylethoxymalonate (1.97 g, 9.13 mmol) was stirred at 100-110°C for one hour to form a homogenous solution. The solution was cooled to room temperature, and pale yellowish crystals were formed. The crystals were collected and washed
15 with hexane, giving 2.33 g (yield 100%) of ethyl-α-carbethoxy-β-(m-cyanoanilino)acrylate (1), mp 109-111°C.

The acrylate ester (1) was added through the top of an air condenser in portions to boiling diphenyl ether (10 ml). After a few minutes of addition, crystals formed. The resulting
20 mixture was heated at the same temperature for 30 minutes and

then the cooled to room temperature. The crystals were collected and washed with hexane, to give 1.79 g of the regioisomer esters (2) (yield 89.5%), mp 305-307°C (dec.).

The esters (2) (1.79 g, 7.39 mmol) were suspended in 10% NaOH (15 ml), and the mixture was heated to reflux for one hour and cooled. Decolorizing charcoal (1.0 g) was added, and the mixture was heated to reflux for an additional 10 minutes. The solid was removed, and the filtrate was acidified to pH 5 with 10% HCl. A cream precipitate was collected, washed with water and hexane, and dried to give the acid isomer mixtures (3), 1.63 g (yield 100%), mp>320°C.

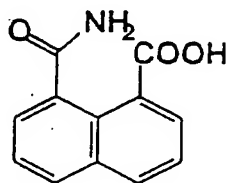
The acids (3) (0.5 g, 2.33 mmol) were added to pre-heated polyphosphoric acid (PPA) (2.2 g) in portions over a period of about 8 minutes with stirring at 255-265°C. The mixture was heated at the same temperature for 20 minutes, cooled to room temperature, and then poured into ice-water. An undesired precipitate, 0.259 g of 7-aminocarbonyl-4-hydroxyisoquinoline, was formed, collected and washed with water (yield 59.1%). The remaining aqueous solution was adjusted to pH 5-6 with NaHCO₃ solution to precipitate out 138 mg of 4-hydroxyquinoline-5-carboxamide (4) (yield 31.5%), mp>250°C.

Compound (4) (1.0 g, 5.88 mmol) was suspended in POCl₃, and the resulting mixture was heated to a temperature of 130°C (bath temperature). After 10 minutes, the suspension became a dark solution, and gases were vigorously released. After one hour and forty-five minutes, the reaction was complete, as shown by thin layer chromatography. The reaction mixture was cooled to room temperature, poured into ice water and basified with 10% NaOH to pH 9. A pale purple precipitate was formed, collected and washed with water. The solid was dissolved in MeOH and decolorized with activated carbon. The solid was removed and the remaining filtrate was evaporated under a vacuum to afford 300 mg (yield 25%) of a white crystalline powder, 4-hydroxyquinoline-5-carboxamide (5), mp 205-207°C.

n-Butyllithium (3.88 mmol) in hexane was added slowly to a solution of 4-hydroxyquinoline-5-carboxamide (5) (400 mg, 1.94 mmol) in THF (10 ml) at a temperature of -78°C. Next, dry CO₂ gas was bubbled into the mixture for 15 minutes at -78°C. Saturated NH₄Cl solution (20 ml) was also added. The mixture

was warmed to room temperature, concentrated, and the remaining residue was dissolved in a saturated Na_2CO_3 solution. The resulting aqueous solution was washed with ether and then acidified to pH 6 with 1N HCl to afford 124 mg of the product 5 compound (6) as a solid.

Additionally, compounds related to 8-carboxy-naphthalene-1-carboxamide (also known as 8-carbamoyl-naphthalene carboxylic acid), shown below:

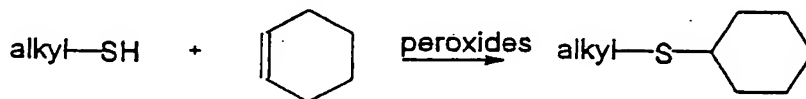


10 can be prepared by known chemical syntheses such as, for example, that described in *Gazz. Chim. Ital.*, 79:603-605 (1949). Moreover, the particular compound shown above is commercially available from Lancaster Synthesis Inc., P.O. Box 1000, Windham, NH 03087, USA.

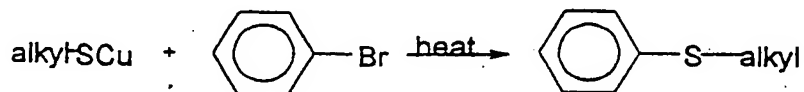
15

Synthesis of Thioalkyl PARP Inhibitors

The usual building blocks for synthesizing organosulfur compounds are alkyl thiols, sometimes called mercaptans, which can be handled much like alcohols. Specifically, thioalkyl 20 compound PARP inhibitors may be prepared through nucleophilic or radical reactions, such as:

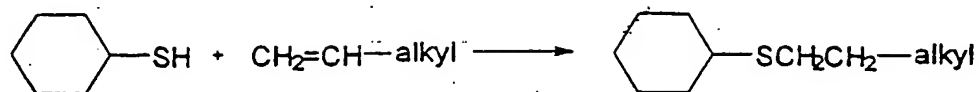


Although direct nucleophilic displacements do not usually occur on simple aryl halides, the reaction to form a thioalkyl 25 compound proceeds readily, for example:

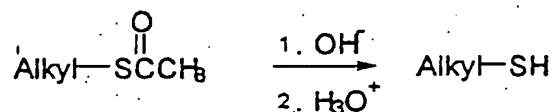


Another synthetic pathway is to form a cyclic thiol, and then use the free-radical addition of an alkene to the thiol to

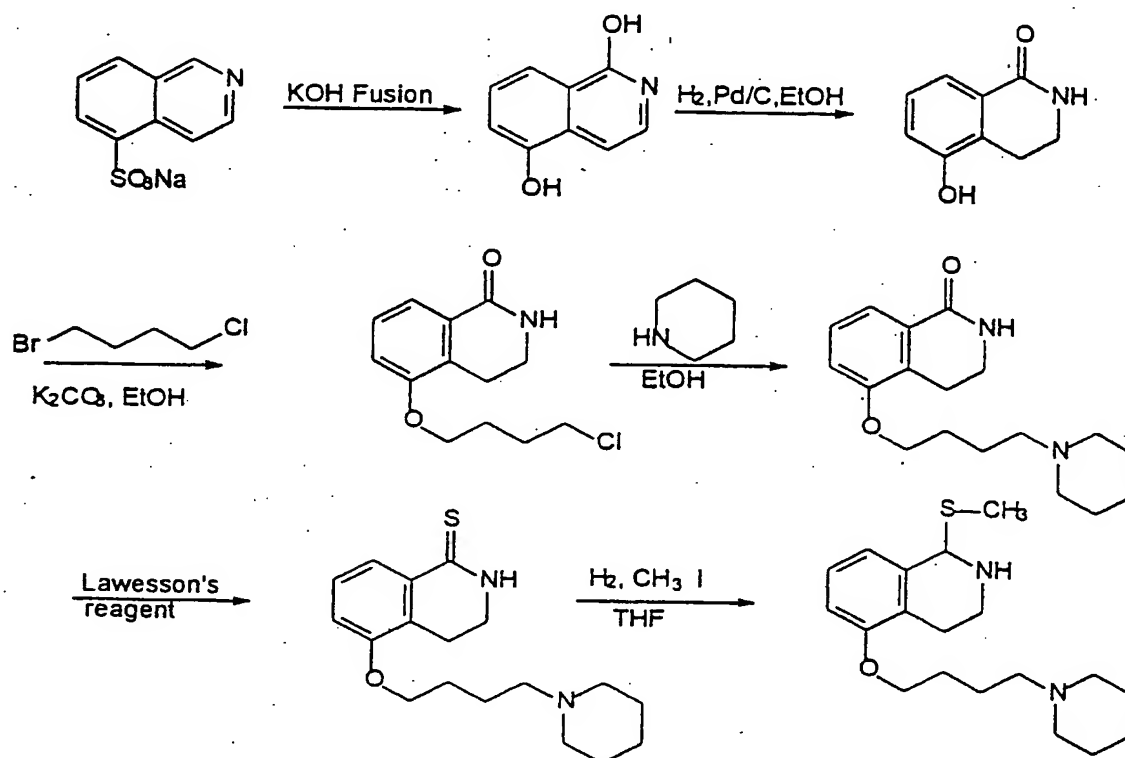
form a thioalkyl PARP inhibitor, as shown below:



Thioalkyls themselves can be readily prepared by the action of sulfur on Grignard reagents or by the hydrolysis of thioalkyl esters, shown below:



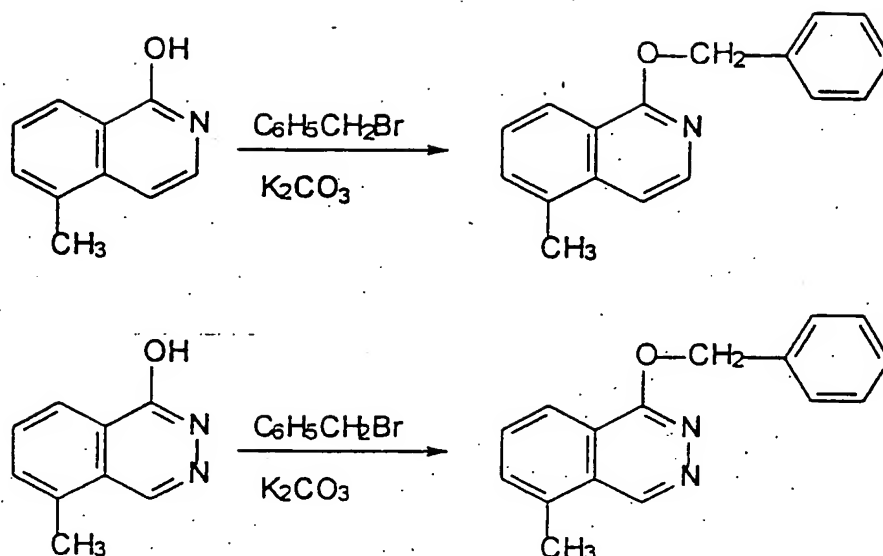
A preferred synthetic pathway for preparing an exemplary thioalkyl PARP inhibitor comprises the following steps:



Synthesis of Alkoxy-substituted PARP Inhibitors

Alkoxy-substituted PARP inhibitors may be prepared by reacting an alkoxide with a primary alkyl halide to yield an ether by an S_N2 pathway, a process known as the Williamson ether synthesis. Specifically, an alkoxide anion is reacted with RX , wherein X is bromo, chloro or iodo. The alkoxide anion needed for the Williamson reaction to proceed is typically generated by reacting an alcohol with a strong base, such as sodium hydride, $NaOH$, KOH , K_2CO_3 , Na_2CO_3 , n -butyllithium or the like. The resulting acid-base reaction produces the intermediate anion for reaction with the halide RX .

Specific examples of this reaction include:



Typically, the reactions shown above take place in a solvent that is inert with respect to both the alkoxide anion and RX and that allows at least some of the RX to go into solution. Typical solvents include, for example, methylene chloride, chloroform, tetrahydrofuran, dimethylformamide, and a variety of other inert organic solvents.

The above-described reaction can take place at varying temperatures depending, for example, upon the solvent used, the solubility of the alkoxide anion and RX in the solvent being used, and the susceptibility of the reactions to oxidize or participate in side reactions. Preferably, however, when the above reaction is used, it takes place at a temperature from about $0^{\circ}C$ to about $100^{\circ}C$, preferably at about room temperature.

The time required for the above reaction also can vary widely, depending on much the same factors. Typically, however, the reaction takes place within a time of about 5 minutes to about 24 hours, preferably from about 10 minutes to about two hours.

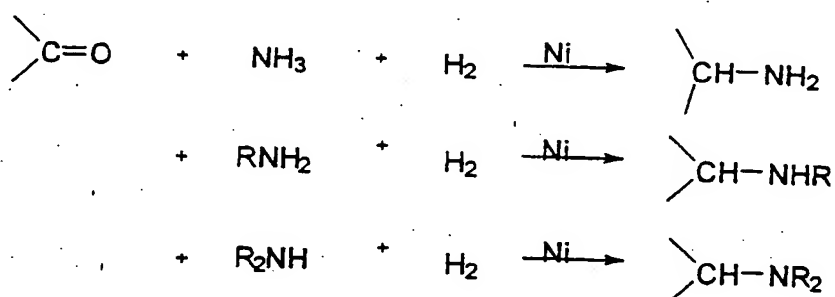
The addition sequence of the alcohol, the base, a solvent (if used), and the RX compound, can vary significantly depending upon the relative reactivities of these materials, the purity of these materials, the temperature at which the reaction is performed, the degree of agitation used in the reaction, and the like. Preferably, however, the alkoxide

anion intermediate is first dissolved in a solvent, the base is added, and then the RX compound is added.

Synthesis of Amino-substituted Parp Inhibitors

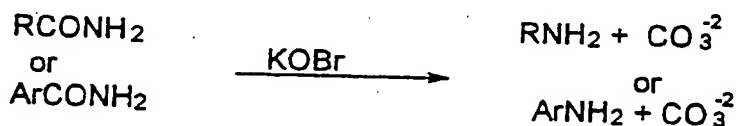
5 Some of the many general methods used to prepare amines are:

- (1) Reduction of corresponding nitro compounds, especially to produce aromatic amines;
- (2) Reaction of halides with ammonia and amines, especially
10 where the halide is an alkyl group or an aryl group having electron-withdrawing substituents;
- (3) Reductive amination of the corresponding ketone to form primary, secondary or tertiary amines, such as shown below:

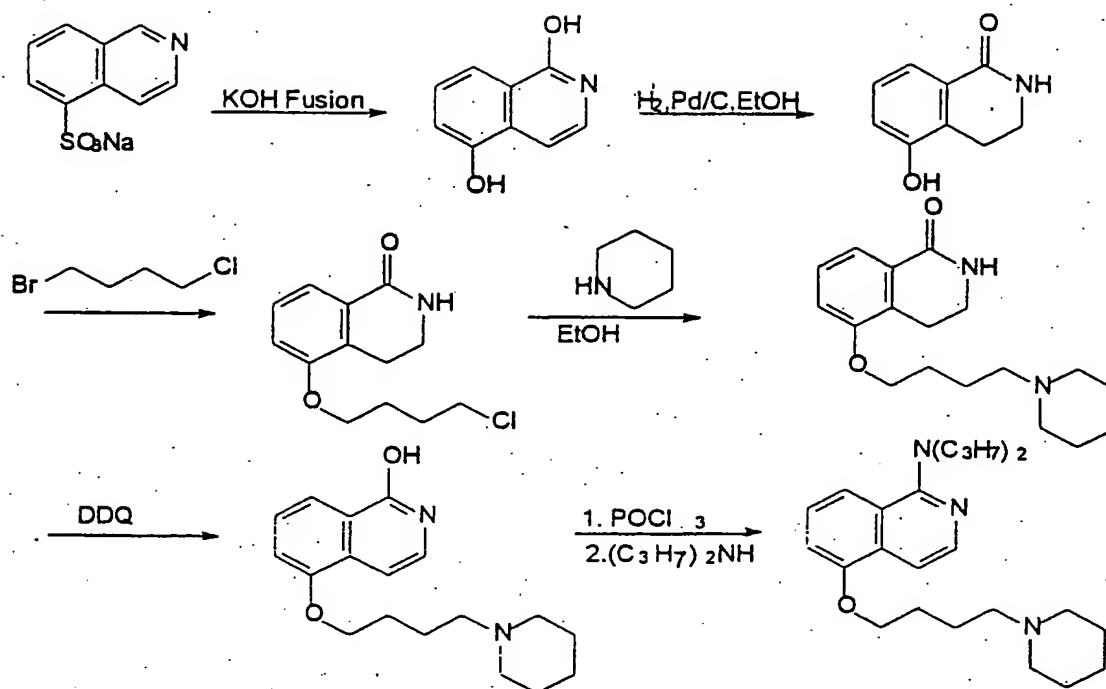


15 The above reactions typically occur in the presence of methanol or ethanol and a reducing agent such as NaBH_3CN .

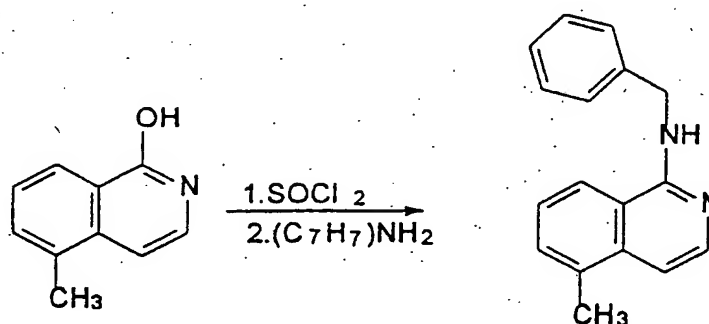
- (4) Reduction of corresponding nitriles; and
- (5) Hoffman degradation of amides, such as shown below:



20 A procedure for the preparation of an exemplary amino-substituted PARP inhibitor follows:



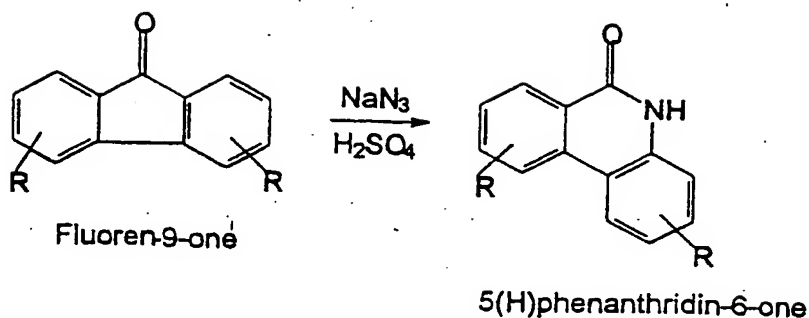
As indicated below, the last step in the above reaction may also be performed with thionyl chloride (SOCl_2) instead of phosphorus oxychloride (POCl_3):



5

Synthesis of Oxo-substituted PARP Inhibitors

Phenanthridinones are the preferred building blocks for synthesizing multicyclic, nitrogen-containing PARP inhibitors that are substituted by double-bonded oxygen. The Schmidt method can be used in a conventional manner to make generically substituted (5H)-phenanthridin-6-one PARP inhibitors as illustrated below:



Phenanthridinones can also be prepared through an intramolecular Heck reaction analogous to that disclosed by Chide et al., *Tetrahedron Lett.*, 32:35, 4525-28 (1991).

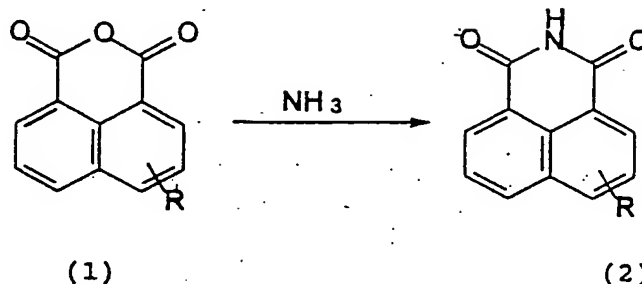
5 Other methods that may be useful in preparing oxo-substituted PARP inhibitors include, but are not limited to:

- I. the Smith reaction of Responddy et al., *Acad. Sci. Paris, Ser. C*, (1967);
- II. the photocyclization method described by Ninomiya et al., *Tetrahedron Lett.*, 4451 (1970) and Ichiya et al., *J. Chem. Soc.*, 1:2257 (1973);
- 10 III. isocyanate intramolecular cycloaddition reactions, such as that found in:
 - (a) Balazs et al., *Synthesis*, 1373 (1995)); Banwell et al., *J. Chem. Soc.*, 1:3515 (1994);
 - 15 (b) Migachev et al., *J. Org. Chem. USSR (Eng. Trans.)*, 20:8, 1565-71 (1984) and *Zh. Org. Khim.*, 20:8, 1718-24 (1984);
 - (c) Migachev et al., *Chem. Heterocycl. Compd. (Eng. Trans.)*, 17:3, 289-94 (1981) and *Khim. Geterotsikl. Soedin.*, 17:3, 388-91 (1981);
 - 20 (d) Migatschew et al., *J. Gen. Chem. USSR (Eng. Trans.)*; 48, 2116, (1978));
 - (e) Chandler et al., *Aust. J. Chem.*, 20, 2037-44 (1967));
 - 25 (f) Ruediger et al., *Can. J. Chem.*, 64, 577-9 (1986).

Synthesis of Tricyclic PARP Inhibitors

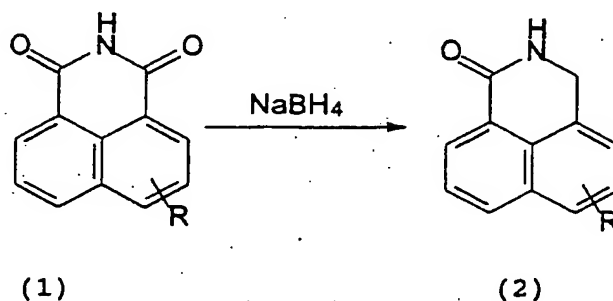
A procedure for the preparation of an exemplary tricyclic, nitrogen-containing PARP inhibitor, R-substituted 1H-

benzo[de]iso-quinoline-1,3(2H)-dione, is shown below:



5 The starting R-substituted 1,8-naphthalic anhydride may be purchased from commercial sources or may be known in the chemistry literature and accessible by processes known to one skilled in the art. To a solution of R-substituted 1,8-naphthalic anhydride (1) (10 mmol) in ethanol (100 ml), ammonia
10 is introduced at a temperature of 40°C. After about five minutes, the ammonia gas line is withdrawn and the mixture is stirred continuously at 50°C for two hours. The ethanol solvent and excess ammonia are removed in vacuo. The resulting residue is purified either by crystallization or by column
15 chromatography on silica gel to give the desired 1H-benzo[de]isoquinoline-1,3(2H)-dione (2), which appears as essentially colorless crystals.

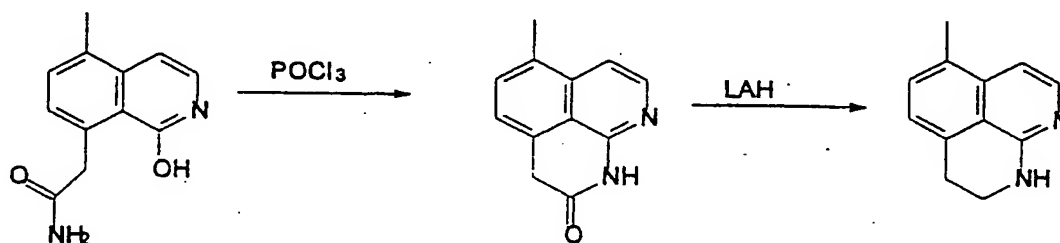
Another procedure for the preparation of an exemplary tricyclic, nitrogen-containing PARP inhibitor, R-substituted
20 2,3,3a,9b-tetrahydro-1H-benzo[de]isoquinolin-1-one, is shown below:



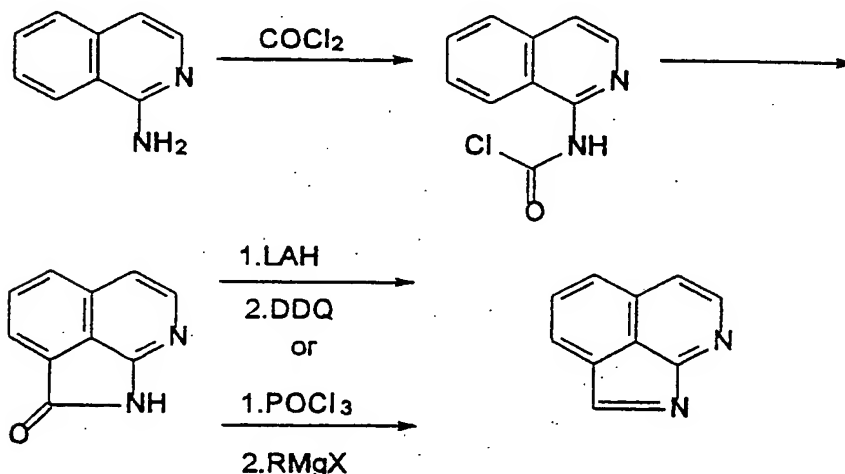
To a solution of sodium borohydride (5 mmol) in
25 ethanol/water (20 ml, v/v:10/1), the R-substituted 1H-benzo[de]isoquinoline-1,3(2H)-dione (0.5 mmol), obtained from Example 1 above is added. The resulting mixture is stirred for four hours at 60°C. After quenching the reaction with 2N hydrochloric acid, the reaction mixture is extracted with

methylene chloride (30 ml x 3). The organic layers over combined and dried over anhydrous sodium sulfate. The solvent is then removed leaving a solid residue. The residue is purified either by crystallization or by column chromatography 5 on silica gel, to give the desired compound, a R-substituted 2,3,3a,9b-tetrahydro-1H-benzo[de]iso-quinolin-1-one (2), which appears as essentially colorless crystals.

The following are some additional methods for preparation of exemplary tricyclic, nitrogen-containing PARP inhibitors:



10



The products of each of the above syntheses for each type of PARP inhibitor are isolated from their respective reaction 15 mixtures by conventional techniques, such as by precipitating out, extraction with an immiscible solvent under appropriate pH conditions, evaporation, filtration, crystallization, or by column chromatography on silica gel and the like. Typically, however, the products are removed either by crystallization or 20 column chromatography on silica gel.

Other variations and modifications of this invention using the synthetic pathways described above will be obvious to those

skilled in the art. Precursor compounds can be prepared by methods known in the art. See, for example, L. Paquette, *Principles of Modern Heterocyclic Chemistry* (1968).

Typically, the PARP inhibitors used in the composition of 5 the invention will have an IC_{50} for inhibiting poly(ADP-ribose) polymerase in vitro of 100 μ M or lower, preferably 25 μ M or lower, more preferably 12 μ M or lower and, even more preferably, 12 mM or lower.

10 Pharmaceutical Compositions

The present invention further relates to a pharmaceutical composition comprising:

- (i) an effective amount of a compound which inhibits PARP activity, treats tissue damage resulting from cell damage or 15 death due to necrosis or apoptosis, or effects a neuronal activity not mediated by NMDA toxicity; and
- (ii) a pharmaceutically acceptable carrier.

The neuronal activity may be selected from the group consisting of stimulation of damaged neurons, promotion of 20 neuronal regeneration, prevention of neurodegeneration, and treatment of a neurological disorder.

Preferably, the neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical 25 damage to the spinal cord, stroke, demyelinating disease and neurological disorder relating to neurodegeneration.

A preferred neurological disorder is stroke, and a preferred neurological disorder relating to neurodegeneration is selected from the group consisting of Alzheimer's Disease, 30 Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis.

The compound of the pharmaceutical composition of the invention may interact with PARP by forming at least one hydrogen bond with an amino acid in PARP, potentially Ser 35 and/or Gly. Specifically, an NH group of the compound may form a hydrogen bond with the O atom of Gly in PARP. The length of such bond is about 1-5 Å, preferably about 2-4 Å. Additionally, an O, S or N atom of the compound may form a hydrogen bond with the H atom of Ser. The length of such bond

is about 1-5 Å, preferably about 2-4 Å.

In an additional preferred embodiment of the invention, the compound of the composition exhibits an IC_{50} for inhibiting PARP in vitro of about 100 uM or lower, more preferably, about 5 25 uM or lower.

An additional embodiment, a encompasses the compounds described herein, compositions containing the same, and methods of using the same including methods of inhibiting PARP activity by administering a compound, as described above. In yet 10 further embodiments, the amount of the compound administered in the methods of the invention is sufficient for treating tissue damage resulting from cell damage or death due to necrosis or apoptosis, neural tissue damage resulting from ischemia and reperfusion injury, or neurological disorders and 15 neurodegenerative diseases; to prevent or treat vascular stroke; to treat or prevent cardiovascular disorders; to treat other conditions and/or disorders such as age-related macular degeneration, AIDS and other immune senescence diseases, arthritis, atherosclerosis, cachexia, cancer, degenerative 20 diseases of skeletal muscle involving replicative senescence, diabetes, head trauma, immune senescence, inflammatory bowel disorders (such as colitis and Crohn's disease), muscular dystrophy, osteoarthritis, osteoporosis, chronic and/or acute pain (such as neuropathic pain), renal failure, retinal 25 ischemia, septic shock (such as endotoxic shock), and skin aging; to extend the lifespan and proliferative capacity of cells; to alter gene expression of senescent cells; or to radiosensitize hypoxic tumor cells.

In another preferred embodiment of the pharmaceutical 30 composition of the invention, the compound is present in an amount that is effective for treating a cardiovascular disorder in an animal. The cardiovascular disorder may be selected from the group consisting of coronary artery disease, angina pectoris, myocardial infarction, cardiac arrest, cardiogenic 35 shock, and cardiovascular tissue damage.

In still another preferred embodiment of the pharmaceutical composition of the invention, the compound is present in an amount that is effective for treating organ damage due to transplantation.

In an additional preferred embodiment of the pharmaceutical composition of the invention, the compound is present in an amount that is effective for treating an inflammatory bowel disorder in an animal. Preferably, the
5 inflammatory bowel disorder is Crohn's Disease or colitis.

In still another preferred embodiment of the pharmaceutical composition of the invention, the compound is present in an amount that is effective for treating arthritis in an animal.

10 In a further preferred embodiment of the pharmaceutical composition of the invention, the compound is present in an amount that is effective for treating diabetes in an animal.

In yet another preferred embodiment of the pharmaceutical composition of the invention, the compound is present in an
15 amount that is effective for treating septic shock in an animal. In another preferred embodiment, the type of septic shock is endotoxic shock or acute tubular necrosis.

In still another preferred embodiment of the present invention, the pharmaceutical composition contains the compound
20 in an amount effective for treating tissue damage resulting from cell damage or death due to necrosis or apoptosis and diseases and conditions relating thereto including, but not limited to, renal failure, cachexia, retinal ischemia, skin aging, atherosclerosis, osteoarthritis, osteoporosis, chronic
25 pain, acute pain, neuropathic pain, muscular dystrophy, degenerative diseases of skeletal muscle involving replicative senescence, age-related macular degeneration, immune senescence, AIDS and other immune senescence diseases, and cancer.

30 In further embodiments of the present invention, the compound in the pharmaceutical composition is present in amounts sufficient to extend the lifespan and proliferative capacity of cells; and/or to alter gene expression of senescent cells; and/or to radiosensitize hypoxic tumor cells.

35 Finally, in another preferred embodiment of the pharmaceutical composition of the invention, the compound is present in an amount that is effective for treating cancer in an animal. The cancer may be selected from the group consisting of ACTH-producing tumors, acute lymphocytic

leukemia, acute nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, 5 endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, 10 mesothelioma, multiple myeloma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma, ovarian cancer, ovarian (germ cell) cancer, pancreatic cancer, penile cancer, prostate cancer, retinoblastoma, skin cancer, soft tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, 15 trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva, and Wilm's tumor.

The compounds of the invention are useful in the manufacture of pharmaceutical formulations comprising an effective amount thereof in conjunction with or as an admixture 20 with excipients or carriers suitable for either enteral or parenteral application. As such, formulations of the present invention suitable for oral administration may be in the form of discrete units such as capsules, cachets, tablets, troche or lozenges, each containing a predetermined amount of the active 25 ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or nonaqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion. The active ingredient may also be in the form of a bolus, electuary, or paste. Each formulation may 30 contain from about 0.01% to about 99.99% by weight, preferably from about 3.5% to about 60% by weight, of the compound of the invention, as well as one or more pharmaceutical excipients, such as wetting, emulsifying and pH buffering agents.

The composition will usually be formulated into a unit 35 dosage form, such as a tablet, capsule, aqueous suspension or solution. Such formulations typically include a solid, semisolid, or liquid carrier. Exemplary carriers include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, mineral oil, cocoa butter, oil of

theobroma, alginates, tragacanth, gelatin, syrup, methyl cellulose, polyoxyethylene sorbitan monolaurate, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, corn starch and the like.

5 Particularly preferred formulations include tablets and gelatin capsules comprising the active ingredient together with (a) diluents, such as lactose, dextrose, sucrose, mannitol, sorbitol, cellulose, dried corn starch, and glycine; and/or (b) lubricants, such as silica, talcum, stearic acid, its magnesium
10 or calcium salt, and polyethylene glycol.

Tablets may also contain binders, such as magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and polyvinylpyrrolidone; disintegrants, such as starches, agar,
15 alginic acid or its sodium salt, and effervescent mixtures; and/or absorbents, colorants, flavors, and sweeteners. The compositions of the invention may be sterilized and/or contain adjuvants, such as preserving, stabilizing, swelling or emulsifying agents, solution promoters, salts for regulating
20 osmotic pressure, and/or buffers. In addition, the composition may also contain other therapeutically valuable substances. Aqueous suspensions may contain emulsifying and suspending agents combined with the active ingredient. All oral dosage forms may further contain sweetening and/or flavoring and/or
25 coloring agents.

These compositions are prepared according to conventional mixing, granulating, or coating methods, respectively, and contain about 0.1 to 75% of the active ingredient, preferably about 1 to 50% of the same. A tablet may be made by
30 compressing or molding the active ingredient optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent,
35 surface active, or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered active ingredient and a suitable carrier moistened with an inert liquid diluent.

When administered parenterally, the composition will

normally be in a unit dosage, sterile injectable form (aqueous isotonic solution, suspension or emulsion) with a pharmaceutically acceptable carrier. Such carriers are preferably non-toxic, parenterally-acceptable and contain non-therapeutic diluents or solvents. Examples of such carriers include water; aqueous solutions, such as saline (isotonic sodium chloride solution), Ringer's solution, dextrose solution, and Hanks' solution; and nonaqueous carriers, such as 1,3-butanediol, fixed oils (e.g., corn, cottonseed, peanut, sesame oil, and synthetic mono- or di-glyceride), ethyl oleate, and isopropyl myristate.

Oleaginous suspensions can be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. Among the acceptable solvents or suspending mediums are sterile fixed oils. For this purpose, any bland fixed oil may be used. Fatty acids, such as oleic acid and its glyceride derivatives, including olive oil and castor oil, especially in their polyoxyethylated forms, are also useful in the preparation of injectables. These oil solutions or suspensions may also contain long-chain alcohol diluents or dispersants.

Sterile saline is a preferred carrier, and the compounds are often sufficiently water soluble to be made up as a solution for all foreseeable needs. The carrier may contain minor amounts of additives, such as substances that enhance solubility, isotonicity, and chemical stability, e.g., anti-oxidants, buffers and preservatives.

When administered rectally, the composition will usually be formulated into a unit dosage form such as a suppository or cachet. These compositions can be prepared by mixing the compound with suitable non-irritating excipients that are solid at room temperature, but liquid at rectal temperature, such that they will melt in the rectum to release the compound. Common excipients include cocoa butter, beeswax and polyethylene glycols or other fatty emulsions or suspensions.

Moreover, the compounds may be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin or the

lower intestinal tract.

For topical application to the eye, or ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH-adjusted sterile saline or, preferably, as a solution in isotonic, pH-adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, the compounds may be formulated into ointments, such as petrolatum.

For topical application to the skin, the compounds can be formulated into suitable ointments containing the compounds suspended or dissolved in, for example, mixtures with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene compound, polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated into suitable lotions or creams containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl ester wax, cetaryl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application to the lower intestinal tract can be effected in rectal suppository formulations (see above) or in suitable enema formulations.

Formulations suitable for nasal or buccal administration, (such as self-propelling powder dispensing formulations), may comprise about 0.1% to about 5% w/w of the active ingredient or, for example, about 1% w/w of the same. In addition, some formulations can be compounded into a sublingual troche or lozenge.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient into association with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active ingredient into association with a liquid carrier or a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation.

In a preferred embodiment, the carrier is a solid biodegradable polymer or mixture of biodegradable polymers with appropriate time release characteristics and release kinetics. The composition of the invention may then be molded into a solid implant suitable for providing efficacious concentrations of the compounds of the invention over a prolonged period of time without the need for frequent redosing. The composition of the present invention can be incorporated into the biodegradable polymer or polymer mixture in any suitable manner known to one of ordinary skill in the art and may form a homogeneous matrix with the biodegradable polymer, or may be encapsulated in some way within the polymer, or may be molded into a solid implant. In one embodiment, the biodegradable polymer or polymer mixture is used to form a soft "depot" containing the pharmaceutical composition of the present invention that can be administered as a flowable liquid, for example, by injection, but which remains sufficiently viscous to maintain the pharmaceutical composition within the localized area around the injection site. The degradation time of the depot so formed can be varied from several days to a few years, depending upon the polymer selected and its molecular weight. By using a polymer composition in injectable form, even the need to make an incision may be eliminated. In any event, a flexible or flowable delivery "depot" will adjust to the shape of the space it occupies with the body with a minimum of trauma to surrounding tissues. The pharmaceutical composition of the present invention is used in amounts that are therapeutically effective and the amounts used may depend upon the desired release profile, the concentration of the pharmaceutical composition required for the sensitizing effect, and the length of time that the pharmaceutical composition has to be released for treatment.

The composition of the invention is preferably administered as a capsule or tablet containing a single dose or a series of divided doses of the compound, or as a sterile solution, suspension, or emulsion, for parenteral administration in a single or divided dose.

In another preferred embodiment, the compounds of the invention can be prepared in lyophilized form. In this case,

1 to 100 mg of a PARP inhibitor may be lyophilized in individual vials, together with a carrier and a buffer, such as mannitol and sodium phosphate. The composition may then be reconstituted in the vials with bacteriostatic water before
5 administration.

The compounds of the invention are used in the composition in amounts that are therapeutically effective. While the effective amount of the PARP inhibitor will depend upon the particular compound being used, amounts of the these compounds
10 varying from about 1% to about 65% have been easily incorporated into liquid or solid carrier delivery systems.

Methods of the Invention

The present invention further relates to a method for
15 treating or preventing tissue damage resulting from cell damage or death due to necrosis or apoptosis and/or a method of effecting a neuronal activity not mediated by NMDA toxicity in an animal, comprising administering to the animal an effective amount of a compound which inhibits PARP activity.

20 The neuronal activity may be selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration, and treatment of a neurological disorder.

Preferably, the neurological disorder is selected from the
25 group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke, demyelinating disease and neurological disorder relating to neurodegeneration.

A preferred neurological disorder is stroke, and a
30 preferred neurological disorder relating to neurodegeneration is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis.

The present invention also relates to a method of treating
35 a cardiovascular disorder in an animal, comprising administering to the animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity. The cardiovascular disorder may be selected from the group consisting of coronary artery

disease, angina pectoris, myocardial infarction, cardiac arrest, cardiogenic shock, and cardiovascular tissue damage.

The present invention also relates to a method of treating organ damage due to transplantation in an animal, comprising
5 administering to the animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

The present invention further relates to a method of treating an inflammatory bowel disorder in an animal,
10 comprising administering to the animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity. Preferably, the bowel disorder treated by the method is Crohn's Disease or colitis.

The present invention also relates to a method of treating
15 arthritis in an animal, comprising administering to the animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

The present invention further relates to a method of treating diabetes in an animal, comprising administering to the
20 animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

The present invention additionally relates to a method of treating septic shock in an animal, comprising administering to
25 the animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity. Preferably, the type of septic shock treated is endotoxic shock.

The invention also relates to a method of treating cancer
30 in an animal, comprising administering to the animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity. The invention further relates to radiosensitizing tumor cells. The type of cancer or tumor cells may be selected from the group
35 consisting of ACTH-producing tumors, acute lymphocytic leukemia, acute nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma,

endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and non-small cell), malignant
5 peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma, ovarian cancer, ovarian (germ cell) cancer, pancreatic cancer, penile cancer, prostate cancer, retinoblastoma, skin cancer, soft tissue sarcoma, squamous cell
10 carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva, and Wilm's tumor.

The term "radiosensitizer", as used herein, is defined as a molecule, preferably a low molecular weight molecule,
15 administered to animals in therapeutically effective amounts to increase the sensitivity of the cells to be radiosensitized to electromagnetic radiation and/or to promote the treatment of diseases which are treatable with electromagnetic radiation. Diseases which are treatable with electromagnetic radiation
20 include neoplastic diseases, benign and malignant tumors, and cancerous cells. Electromagnetic radiation treatment of other diseases not listed herein are also contemplated by the present invention. The terms "electromagnetic radiation" and "radiation" as used herein includes, but is not limited to,
25 radiation having the wavelength of 10^{-20} to 10^2 meters. Preferred embodiments of the present invention employ the electromagnetic radiation of: gamma-radiation (10^{-20} to 10^{-11} m), x-ray radiation (10^{-11} to 10^{-8} m), ultraviolet light (10 nm to 400 nm), visible light (400 nm to 700 nm), infrared radiation
30 (700 nm to 1.0 mm), and microwave radiation (1 mm to 30 cm).

Radiosensitizers are known to increase the sensitivity of cancerous cells to the toxic effects of electromagnetic radiation. Several mechanisms for the mode of action of radiosensitizers have been suggested in the literature
35 including: hypoxic cell radiosensitizers (e.g., 2-nitroimidazole compounds, and benzotriazine dioxide compounds) promote the reoxygenation of hypoxic tissue and/or catalyze the generation of damaging oxygen radicals; non-hypoxic cell radiosensitizers (e.g., halogenated pyrimidines) can be analogs

of DNA bases and preferentially incorporate into the DNA of cancer cells and thereby promote the radiation-induced breaking of DNA molecules and/or prevent the normal DNA repair mechanisms; and various other potential mechanisms of action
5 have been hypothesized for radiosensitizers in the treatment of disease.

Many cancer treatment protocols currently employ radiosensitizers activated by the electromagnetic radiation of x-rays. Examples of x-ray activated radiosensitizers include,
10 but are not limited to, the following: metronidazole, misonidazole, desmethylnisonidazole, pimonidazole, etanidazole, nimorazole, mitomycin C, RSU 1069, SR 4233, EO9, RB 6145, nicotinamide, 5-bromodeoxyuridine (BUdR), 5-iododeoxyuridine (IUdR), bromodeoxycytidine, fluorodeoxyuridine (FudR),
15 hydroxyurea, cisplatin, and therapeutically effective analogs and derivatives of the same.

Photodynamic therapy (PDT) of cancers employs visible light as the radiation activator of the sensitizing agent. Examples of photodynamic radiosensitizers include the
20 following, but are not limited to: hematoporphyrin derivatives, Photofrin, benzoporphyrin derivatives, NPe6, tin etioporphyrin SnET2, pheoborbide-a, bacteriochlorophyll-a, naphthalocyanines, phthalocyanines, zinc phthalocyanine, and therapeutically effective analogs and derivatives of the same.

25 Radiosensitizers may be administered in conjunction with a therapeutically effective amount of one or more other compounds, including but not limited to: compounds which promote the incorporation of radiosensitizers to the target cells; compounds which control the flow of therapeutics,
30 nutrients, and/or oxygen to the target cells; chemotherapeutic agents which act on the tumor with or without additional radiation; or other therapeutically effective compounds for treating cancer or other disease. Examples of additional therapeutic agents that may be used in conjunction with
35 radiosensitizers include, but are not limited to: 5-fluorouracil, leucovorin, 5'-amino-5'-deoxythymidine, oxygen, carbogen, red cell transfusions, perfluorocarbons (e.g., Fluosol-DA), 2,3-DPG, BW12C, calcium channel blockers, pentoxifylline, antiangiogenesis compounds, hydralazine, and L-

BSO. Examples of chemotherapeutic agents that may be used in conjunction with radiosensitizers include, but are not limited to: adriamycin, camptothecin, carboplatin, cisplatin, daunorubicin, docetaxel, doxorubicin, interferon (alpha, beta, 5 gamma), interleukin 2, irinotecan, paclitaxel, topotecan, and therapeutically effective analogs and derivatives of the same.

The present invention further relates to treating or preventing tissue damage resulting from cell damage or death due to necrosis or apoptosis, and conditions and diseases 10 related thereto including, but not limited to, renal failure, cachexia, retinal ischemia, skin aging, atherosclerosis, osteoarthritis, osteoporosis, chronic pain, acute pain, neuropathic pain, muscular dystrophy, degenerative diseases of skeletal muscle involving replicative senescence, age-related 15 macular degeneration, immune senescence, AIDS and other immune senescence diseases, and cancer; to extend the lifespan and proliferative capacity of cells; to alter gene expression of senescent cells; and to radiosensitize hypoxic tumor cells

The present invention still further relates to method of 20 inhibiting PARP activity in an animal, comprising administering to the animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

The compounds used in each of the above methods of the 25 invention preferably interact with PARP by forming at least one hydrogen bond with Ser and/or Gly in PARP. Specifically, an NH group of the compound preferably forms a hydrogen bond with the O atom of Gly in PARP. The length of such bond is about 1-5 Å, preferably about 2-4 Å. Additionally, an O, S or N atom of the 30 compound preferably forms a hydrogen bond with the H atom of Ser. The length of such bond is about 1-5 Å, preferably about 2-4 Å.

In an additional preferred embodiment, the compound used in each of the above methods of the invention exhibits an IC_{50} 35 for inhibiting PARP in vitro of about 100 μM or lower, more preferably, about 25 μM or lower.

Compositions and Methods for Effecting Neuronal Activity

Preferably, the compounds of the invention inhibit PARP

activity and, thus, are believed to be useful for treating neural tissue damage, particularly damage resulting from cerebral ischemia and reperfusion injury or neurodegenerative diseases in mammals. The term "nervous tissue" refers to the various components that make up the nervous system including, without limitation, neurons, neural support cells, glia, Schwann cells, vasculature contained within and supplying these structures, the central nervous system, the brain, the brain stem, the spinal cord, the junction of the central nervous system with the peripheral nervous system, the peripheral nervous system, and allied structures.

Further, according to the invention, an effective therapeutic amount of the compounds and compositions described above are administered to animals to effect a neuronal activity, particularly one that is not mediated by NMDA neurotoxicity. Such neuronal activity may consist of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration and treatment of a neurological disorder. Accordingly, the present invention further relates to a method of effecting a neuronal activity in an animal, comprising administering an effective amount of the compound of the invention to the animal.

Examples of neurological disorders that are treatable by the method of using the present invention include, without limitation, trigeminal neuralgia; glossopharyngeal neuralgia; Bell's Palsy; myasthenia gravis; muscular dystrophy; amyotrophic lateral sclerosis; progressive muscular atrophy; progressive bulbar inherited muscular atrophy; herniated, ruptured or prolapsed invertebrate disk syndromes; cervical spondylosis; plexus disorders; thoracic outlet destruction syndromes; peripheral neuropathies such as those caused by lead, dapsone, ticks, porphyria, or Guillain-Barré syndrome; Alzheimer's disease; Huntington's Disease and Parkinson's disease. The term "neurodegenerative diseases" includes Alzheimer's disease, Parkinson's disease and Huntington's disease.

"Nervous function" refers to the various functions of the nervous system, which among other things provide an awareness of the internal and external environments of the body, make

possible voluntary and reflex activities between the various structural elements of the organism, and balance the organism's response to environmental changes.

The term "nervous insult" refers to any damage to nervous tissue and any disability or death resulting therefrom. The cause of nervous insult may be metabolic, toxic, neurotoxic, iatrogenic, thermal or chemical, and includes without limitation, ischemia, hypoxia, cerebrovascular accident, trauma, surgery, pressure, mass effect, hemorrhage, radiation, vasospasm, neurodegenerative disease, infection, Parkinson's disease, amyotrophic lateral sclerosis (ALS), myelination/demyelination process, epilepsy, cognitive disorder, glutamate abnormality and secondary effects thereof.

The term "neuroprotective" refers to the effect of reducing, arresting or ameliorating nervous insult, and protecting, resuscitating, or reviving nervous tissue that has suffered nervous insult.

The term "preventing neurodegeneration" includes the ability to prevent neurodegeneration in patients diagnosed as having a neurodegenerative disease or who are at risk of developing a neurodegenerative disease. The term also encompasses preventing further neurodegeneration in patients who are already suffering from or have symptoms of a neurodegenerative disease.

The term "treating" refers to:

- (i) preventing a disease, disorder or condition from occurring in an animal that may be predisposed to the disease, disorder and/or condition, but has not yet been diagnosed as having it;
- (ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and
- (iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

The method of the present invention is particularly useful for treating a neurological disorder selected from the group consisting of: peripheral neuropathy caused by physical injury or disease state; head trauma, such as traumatic brain injury; physical damage to the spinal cord; stroke associated with brain damage, such as vascular stroke associated with hypoxia

and brain damage, focal cerebral ischemia, global cerebral ischemia, and cerebral reperfusion injury; demyelinating diseases, such as multiple sclerosis; and neurological disorders related to neurodegeneration, such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease and amyotrophic lateral sclerosis (ALS).

The term "neural tissue damage resulting from ischemia and reperfusion injury and neurodegenerative diseases" includes neurotoxicity, such as seen in vascular stroke and global and focal ischemia.

Treating Other PARP-Related Disorders

The compounds, compositions and methods of the invention can also be used to treat a cardiovascular disorder in an animal, by administering an effective amount of the compound of formula IV to the animal. For example, the compounds of the invention can treat or prevent cardiovascular tissue damage resulting from cardiac ischemia or reperfusion injury. Reperfusion injury, for instance, occurs at the termination of cardiac bypass procedures or during cardiac arrest when the heart, once prevented from receiving blood, begins to reperfuse.

As used herein, the term "cardiovascular disorders" refers to those disorders that can either cause ischemia or are caused by reperfusion of the heart. Examples include, but are not limited to, coronary artery disease, angina pectoris, myocardial infarction, cardiovascular tissue damage caused by cardiac arrest, cardiovascular tissue damage caused by cardiac bypass, cardiogenic shock, and related conditions that would be known by those of ordinary skill in the art or which involve dysfunction of or tissue damage to the heart or vasculature, especially, but not limited to, tissue damage related to PARP activation.

The term "ischemia" refers to localized tissue anemia due to obstruction of the inflow of arterial blood. Global ischemia occurs when blood flow to the entire brain ceases for a period of time. Global ischemia may result from cardiac arrest. Focal ischemia occurs when a portion of the brain is

deprived of its normal blood supply. Focal ischemia may result from thromboembolytic occlusion of a cerebral vessel, traumatic head injury, edema or brain tumor. Even if transient, both global and focal ischemia can cause widespread neuronal damage.

5

Although nerve tissue damage occurs over hours or even days following the onset of ischemia, some permanent nerve tissue damage may develop in the initial minutes following the cessation of blood flow to the brain. Much of this damage has been attributed to glutamate toxicity and to the secondary consequences of tissue reperfusion, such as the release of vasoactive products by damaged endothelium and the release of cytotoxic products, such as free radicals and leukotrienes, by the damaged tissue. Ischemia can also occur in the heart in myocardial infarction and other cardiovascular disorders in which the coronary arteries have been obstructed as a result of atherosclerosis, thrombi, or spasm.

For example, the methods of the invention are believed to be useful for treating cardiac tissue damage, particularly damage resulting from cardiac ischemia or caused by reperfusion injury in mammals. The methods of the invention are particularly useful for treating cardiovascular disorders selected from the group consisting of: coronary artery disease, such as atherosclerosis; angina pectoris; myocardial infarction; myocardial ischemia and cardiac arrest; cardiac bypass; and cardiogenic shock. The methods of the invention are especially helpful in treating the acute forms of the above cardiovascular disorders.

Further, the methods of the invention can be used to treat arthritis; diabetes; septic shock, such as endotoxic shock; and inflammatory bowel disorders, such as colitis and Crohn's disease; to treat or prevent renal failure, cachexia, retinal ischemia, or chronic pain, acute pain, neuropathic pain; to alter gene expression in senescent cells by increasing expression of young cell specific genes and/or decreasing expression of senescent cell specific genes; and to extend or increase the lifespan or proliferative capacity of cells; and to treat disease or disease conditions induced or exacerbated

by cellular senescence such as skin aging, Alzheimer's disease, atherosclerosis, osteoarthritis, osteoporosis, age-related macular degeneration, muscular dystrophy or other degenerative diseases of skeletal muscle involving replicative senescence, and immune senescence, including diseases, such as AIDS, that result in immune senescence.

Further, the methods of the invention can be used to treat cancer and to radiosensitize tumor cells. The term "cancer" is interpreted broadly. The compounds of the present invention can be "anti-cancer agents", which term also encompasses "anti-tumor cell growth agents" and "anti-neoplastic agents".

For example, the methods of the invention are useful for treating cancers such as ACTH-producing tumors, acute lymphocytic leukemia, acute nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head & neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and/or non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma, ovarian cancer, ovary (germ cell) cancer, prostate cancer, pancreatic cancer, penile cancer, retinoblastoma, skin cancer, soft-tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva and Wilm's tumor.

Administration

For medical use, the amount required of a compound of formula IV to achieve a therapeutic effect will vary according to the particular compound administered, the route of administration, the mammal under treatment, and the particular disorder or disease concerned. A suitable systemic dose of a compound of formula IV for a mammal suffering from, or likely to suffer from, any condition as described herein is typically in the range of about 0.1 to about 100 mg of base per kilogram

of body weight, preferably from about 1 to about 10 mg/kg of mammal body weight. It is understood that the ordinarily skilled physician or veterinarian will readily be able to determine and prescribe the amount of the compound effective for the desired prophylactic or therapeutic treatment.

In so proceeding, the physician or veterinarian may employ an intravenous bolus followed by an intravenous infusion and repeated administrations, as considered appropriate. In the methods of the present invention, the compounds may be administered, for example, orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, sublingually, vaginally, intraventricularly, or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles.

Parenteral includes, but is not limited to, the following examples of administration: intravenous, subcutaneous, intramuscular, intraspinal, intraosseous, intraperitoneal, intrathecal, intraventricular, intrasternal or intracranial injection and infusion techniques, such as by subdural pump. Invasive techniques are preferred, particularly direct administration to damaged neuronal tissue. While it is possible for the compounds of formula IV to be administered alone, it is preferable to provide it as a part of a pharmaceutical formulation.

To be effective therapeutically as central nervous system targets, the compounds used in the methods of the present invention should readily penetrate the blood-brain barrier when peripherally administered. Compounds which cannot penetrate the blood-brain barrier, however, can still be effectively administered by an intraventricular route.

The compounds used in the methods of the present invention may be administered by a single dose, multiple discrete doses or continuous infusion. Since the compounds are small, easily diffusible and relatively stable, they are well suited to continuous infusion. Pump means, particularly subcutaneous or subdural pump means, are preferred for continuous infusion.

For the methods of the present invention, any effective administration regimen regulating the timing and sequence of doses may be used. Doses of the compounds preferably include

pharmaceutical dosage units comprising an efficacious quantity of active compound. By an efficacious quantity is meant a quantity sufficient to inhibit PARP activity and/or derive the desired beneficial effects therefrom through administration of one or more of the pharmaceutical dosage units. In a particularly preferred embodiment, the dose is sufficient to prevent or reduce the effects of vascular stroke or other neurodegenerative diseases.

An exemplary daily dosage unit for a vertebrate host comprises an amount of from about 0.001 mg/kg to about 50 mg/kg. Typically, dosage levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels being about 0.1 mg to about 1,000 mg. The specific dose level for any particular patient will vary depending upon a variety of factors, including the activity of the specific compound employed; the age, body weight, general health, sex, and diet of the patient; the time of administration; the rate of excretion; any combination of the compound with other drugs; the severity of the particular disease being treated; and the form and route of administration. Typically, in vitro dosage-effect results provide useful guidance on the proper doses for patient administration. Studies in animal models can also be helpful. The considerations for determining the proper dose levels are well-known in the art.

In methods of treating nervous insult (particularly acute ischemic stroke and global ischemia caused by drowning or head trauma), the compounds of the invention can be co-administered with one or more other therapeutic agents, preferably agents which can reduce the risk of stroke (such as aspirin) and, more preferably, agents which can reduce the risk of a second ischemic event (such as ticlopidine).

The compounds and compositions can be co-administered with one or more therapeutic agents either (i) together in a single formulation, or (ii) separately in individual formulations designed for optimal release rates of their respective active agent. When the compounds used in the methods of the invention are administered in combination with one or more other therapeutic agents, specific dose levels for those agents will

depend upon considerations such as those identified above for compositions and methods of the invention in general.

For example, Table II below provides known median dosages for selected chemotherapeutic agents that may be administered in combination with the compounds of the invention to such diseases or various cancers.

TABLE II

	CHEMOTHERAPEUTIC AGENT	MEDIAN DOSAGE
10	Asparaginase	10,000 units
	Bleomycin Sulfate	15 units
	Carboplatin	50-450 mg
	Carmustine	100 mg
	Cisplatin	10-50 mg
15	Cladribine	10 mg
	Cyclophosphamide (lyophilized)	100 mg to 2 gm
	Cyclophosphamide (non-lyophilized)	100 mg to 2 gm
	Cytarabine (lyophilized powder)	100 mg to 2 gm
	Dacarbazine	100-200 mg
20	Dactinomycin	0.5 mg
	Daunorubicin	20 mg
	Diethylstilbestrol	250 mg
	Doxorubicin	10-150 mg
	Etidronate	300 mg
25	Etoposide	100 mg
	Floxuridine	500 mg
	Fludarabine Phosphate	50 mg
	Fluorouracil	500 mg to 5 gm
	Goserelin	3.6 mg
30	Granisetron Hydrochloride	1 mg
	Idarubicin	5-10 mg
	Ifosfamide	1-3 gm
	Leucovorin Calcium	50-350 mg
	Leuprolide	3.75-7.5 mg
35	Mechlorethamine	10 mg

	CHEMOTHERAPEUTIC AGENT	MEDIAN DOSAGE
	Medroxyprogesterone	1 gm
	Melphalan	50 gm
	Methotrexate	20 mg to 1 gm
	Mitomycin	5-40 mg
5	Mitoxantrone	20-30 mg
	Ondansetron Hydrochloride	40 mg
	Paclitaxel	30 mg
	Pamidronate Disodium	30-90 mg
	Pegaspargase	750 units
10	Plicamycin	2,500 mcgm
	Streptozocin	1 gm
	Thiotepa	15 mg
	Teniposide	50 mg
	Vinblastine	10 mg
15	Vincristine	1-5 mg
	Aldesleukin	22 million units
	Epoetin Alfa	2,000-10,000 units
	Filgrastim	300-480 mcgm
	Immune Globulin	500 mg to 10 gm
20	Interferon Alpha-2a	3-36 million units
	Interferon Alpha-2b	3-50 million units
	Levamisole	50 mg
	Octreotide	1,000-5,000 mcgm
25	Sargramostim	250-500 mcgm

For the methods of the present invention, any administration regimen regulating the timing and sequence of delivery of the compound can be used and repeated as necessary to effect treatment. Such regimen may include pretreatment and/or co-administration with additional therapeutic agents.

To maximize protection of nervous tissue from nervous insult, the compounds of the invention should be administered to the affected cells as soon as possible. In situations where nervous insult is anticipated, the compounds are advantageously administered before the expected nervous insult. Such

situations of increased likelihood of nervous insult include surgery, such as carotid endarterectomy, cardiac, vascular, aortic, orthopedic surgery; endovascular procedures, such as arterial catheterization (carotid, vertebral, aortic, cardiac, renal, spinal, Adamkiewicz); injections of embolic agents; the use of coils or balloons for hemostasis; interruptions of vascularity for treatment of brain lesions; and predisposing medical conditions such as crescendo transient ischemic attacks, emboli and sequential strokes.

10 Where pre-treatment for stroke or ischemia is impossible or impracticable, it is important to bring the compounds of the invention into contact with the affected cells as soon as possible, either during or after the event. In the time period between strokes, however, diagnosis and treatment procedures
15 should be minimized to save the cells from further damage and death. Therefore, a particularly advantageous mode of administration with a patient diagnosed with acute multiple vascular strokes is by implantation of a subdural pump to deliver the compound(s) of the invention directly to the
20 infarct area of the brain. Even if comatose, it is expected that the patient would recover more quickly than he or she would without this treatment. Moreover, in any conscious state of the patient, it is expected that any residual neurological symptoms, as well as the re-occurrence of stroke, would be
25 reduced.

As to patients diagnosed with other acute disorders believed to be related to PARP activity, such as diabetes, arthritis and Crohn's disease, the compound of the invention should also be administered as soon as possible in a single or
30 divided dose.

Depending on the patient's presenting symptoms and the degree of response to the initial administration of the compound of the invention, the patient may further receive additional doses of the same or different compounds of the
35 invention, by one of the following routes: parenterally, such as by injection or by intravenous administration; orally, such as by capsule or tablet; by implantation of a biocompatible, biodegradable polymeric matrix delivery system comprising the compound; or by direct administration to the infarct area by

insertion of a subdural pump or a central line. It is expected that the treatment would alleviate the disorder, either in part or in its entirety and that fewer further occurrences of the disorder would develop. It also is expected that the patient
5 would suffer fewer residual symptoms.

Where a patient is diagnosed with an acute disorder prior to the availability of the compounds of the invention, the patient's condition may deteriorate due to the acute disorder and become a chronic disorder by the time that the compounds
10 are available. Even when a patient receives a compound of formula IV for the chronic disorder, it is also expected that the patient's condition would stabilize and actually improve as a result of receiving the compound.

15

EXAMPLES

The following examples are illustrative of preferred embodiments of the invention and are not to be construed as limiting the present invention thereto. All polymer molecular weights are mean average molecular weights. All percentages
20 are based on the percent by weight of the final delivery system or formulation, prepared unless otherwise indicated, and all totals equal 100% by weight.

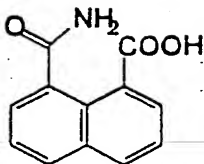
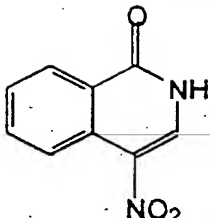
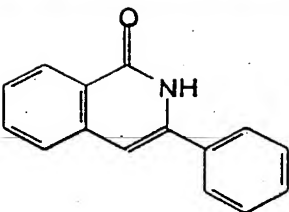
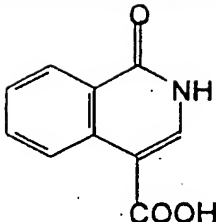
Example 1: Approximate IC₅₀ Data for Selected Compounds

25 The IC₅₀ of with respect to PARP inhibition was determined for several compounds by a PARP assay using purified recombinant human PARP from Trevigen (Gaithersburg, MD), as follows: The PARP enzyme assay was set up on ice in a volume of 100 microliters consisting of 10 mM Tris-HCl (pH 8.0), 1 mM
30 MgCl₂, 28 mM KCl, 28 mM NaCl, 0.1 mg/ml of herring sperm DNA (activated as a 1 mg/ml stock for 10 minutes in a 0.15% hydrogen peroxide solution), 3.0 micromolar [3H]nicotinamide adenine dinucleotide (470 mci/mmol), 7 micrograms/ml PARP enzyme, and various concentrations of the compounds to be
35 tested. The reaction was initiated by incubating the mixture at 25°C. After 15 minutes' incubation, the reaction was terminated by adding 500 microliters of ice cold 20% (w/v) trichloroacetic acid. The precipitate formed was transferred onto a glass fiber filter (Packard Unifilter-GF/B) and washed

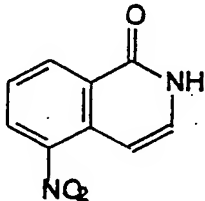
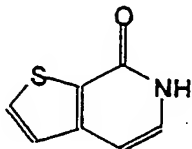
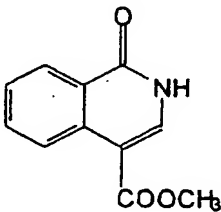
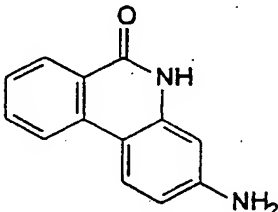
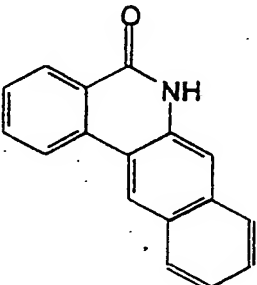
three times with ethanol. After the filter was dried, the radioactivity was determined by scintillation counting.

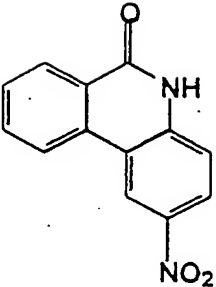
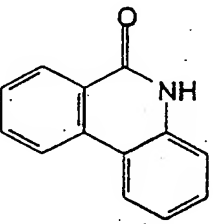
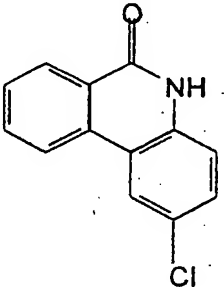
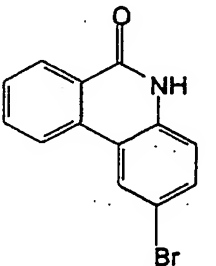
Using the PARP assay described above, approximate IC_{50} values were obtained for the following compounds:

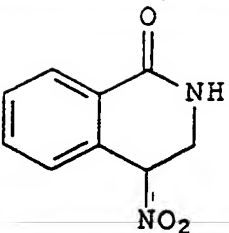
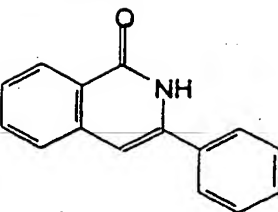
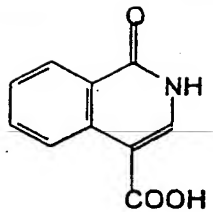
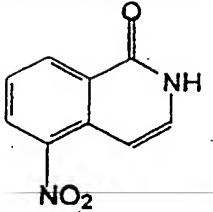
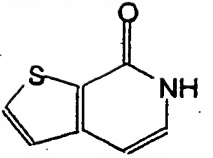
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PARP Inhibitor	Approximate IC_{50} 's
	.25 μM
	5 μM
	30 μM
	10 μM

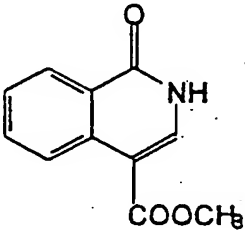
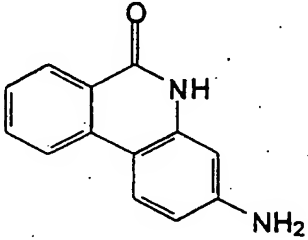
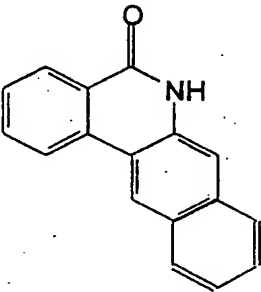
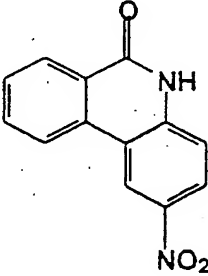
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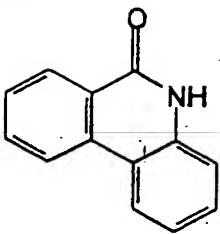
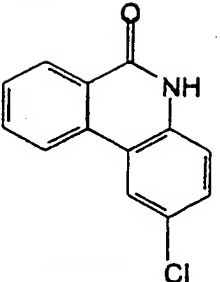
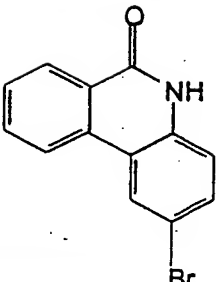
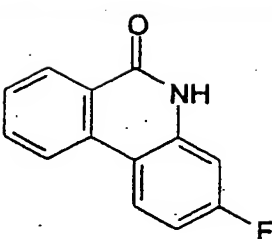
PARP Inhibitor	Approximate IC _{50's}
 <chem>O=C1NC=CC2=CC=CC=C2C1[N+](=O)[O-]</chem>	10 μ M
 <chem>O=C1NC=CC2=CC=CSC2C1</chem>	50 μ M
 <chem>COC(=O)C1=CC2=CC=CC=C2C(=O)NC1</chem>	0.8 μ M
 <chem>NC1=CC=C2C(=C1)C(=O)NC3=CC=CC=C23</chem>	4 μ M
 <chem>O=C1NC2=CC=CC=C2C3=CC=CC=C3C1</chem>	100 μ M

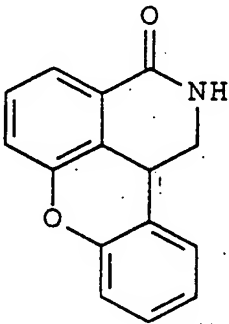
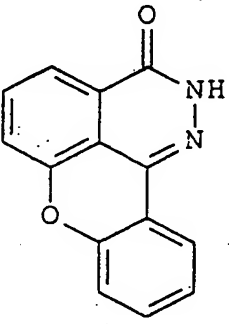
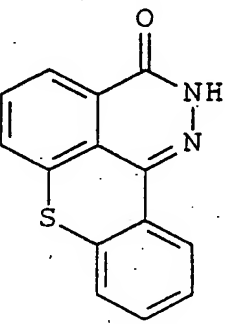
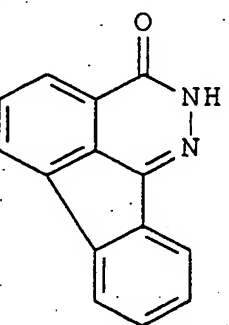
PARP Inhibitor	Approximate IC ₅₀ 's
 <chem>O=C1NC2=CC=C(C=C2C3=CC=CC=C31)C=C(C=C2)C(=O)N</chem>	0.9 μ M
 <chem>O=C1NC2=CC=CC=C2C3=CC=CC=C31</chem>	5.2 μ M
 <chem>O=C1NC2=CC=C(C=C2C3=CC=CC=C31)C=C(C=C2)Cl</chem>	0.7 μ M
 <chem>O=C1NC2=CC=C(C=C2C3=CC=CC=C31)C=C(C=C2)Br</chem>	1.1 μ M

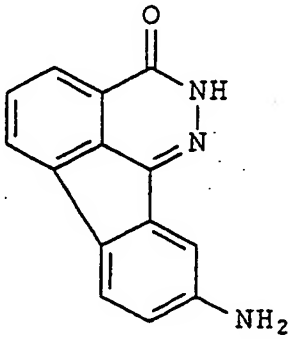
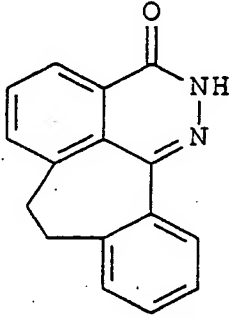
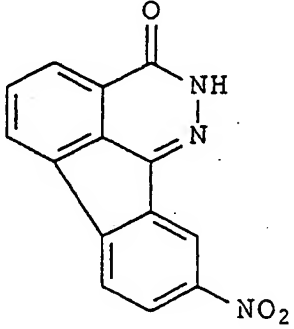
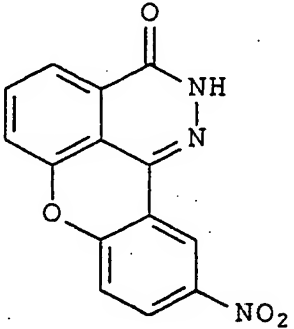
Compound	Approximate IC _{50's}
 <chem>O=C1NC(C(=O)N1)c2ccccc2[N+](=O)[O-]</chem>	1.6 μ M
 <chem>O=C1NC(C1Cc2ccccc2)c3ccccc3</chem>	1.3 μ M
 <chem>O=C1NC(C(=O)N1)c2ccccc2C(=O)O</chem>	10 μ M
 <chem>O=C1NC=Cc2ccccc2[N+](=O)[O-]</chem>	3.4 μ M
 <chem>O=C1NC=Cc2ccsc21</chem>	50 μ M

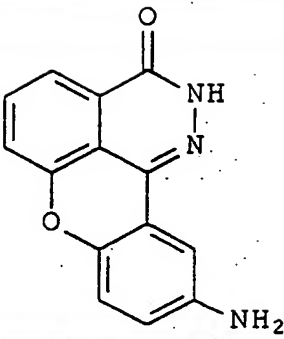
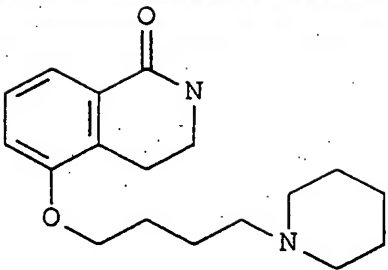
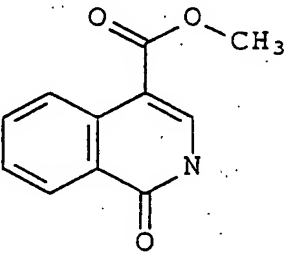
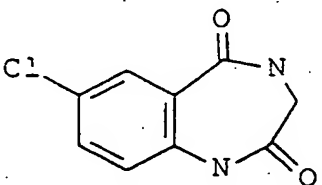
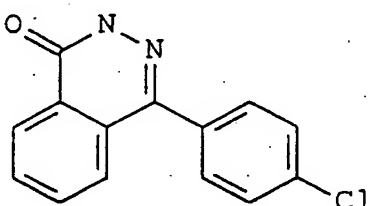
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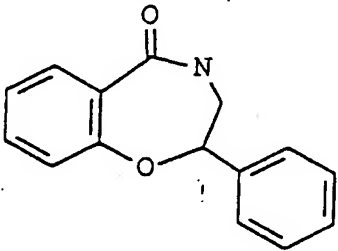
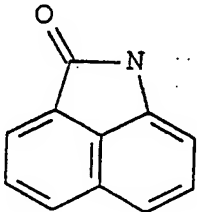
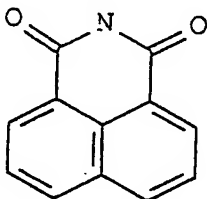
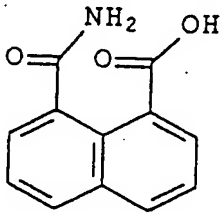
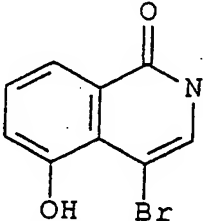
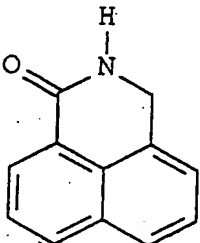
Compound	Approximate IC _{50's}
 <chem>COC(=O)C1=CC(=O)NC2=CC=CC=C12</chem>	0.8 μ M
 <chem>NC1=CC=C2C(=C1)C(=O)NC3=CC=CC=C23</chem>	4 μ M
 <chem>C1=CC=C2C(=C1)C(=O)NC3=CC=CC=C23</chem>	100 μ M
 <chem>O=[N+]([O-])c1ccc2c(c1)C(=O)NC3=CC=CC=C23</chem>	0.9 μ M

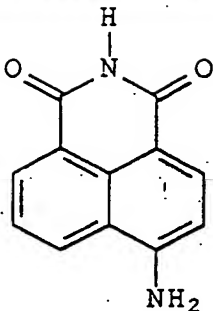
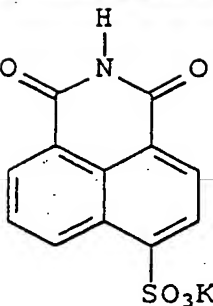
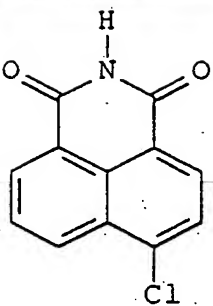
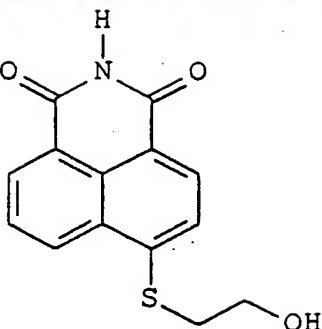
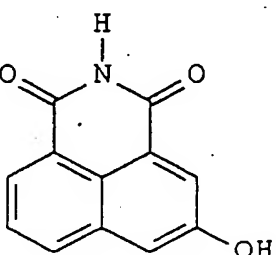
Compound	Approximate IC_{50} 's
 <chem>O=C1c2ccccc2C3=CC=CC=C13</chem>	5.2 μ M
 <chem>O=C1c2ccccc2C3=CC=C(Cl)C=C13</chem>	0.7 μ M
 <chem>O=C1c2ccccc2C3=CC=C(Br)C=C13</chem>	1.1 μ M
 <chem>O=C1c2ccccc2C3=CC=C(F)C=C13</chem>	0.2 μ M

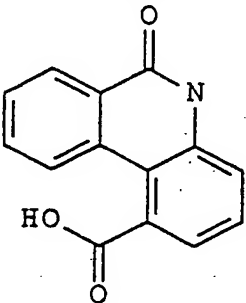
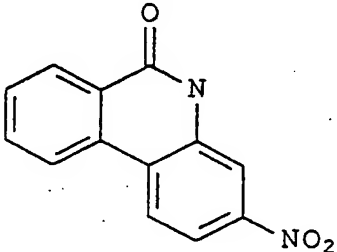
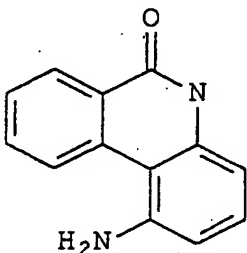
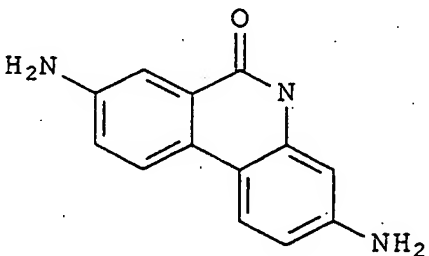
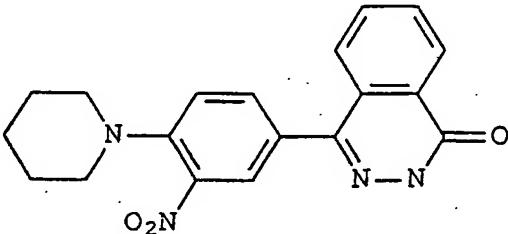
Compound	Approximate IC ₅₀ 's
	0.20
	0.08
	0.11
	0.14

Compound	Approximate IC _{50's}
 <chem>Nc1ccc2c3c(c1)c4ccccc4c(=O)nn32</chem>	0.068
 <chem>O=C1N=NC2=C1C3=CC=CC=C3C4=CC=CC=C24</chem>	0.4
 <chem>[O-][N+](=O)c1ccc2c3c(c1)c4ccccc4c(=O)nn32</chem>	0.056
 <chem>[O-][N+](=O)c1ccc2c3c(c1)c4ccccc4c(=O)nn32O</chem>	0.062

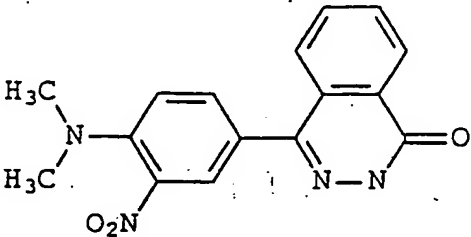
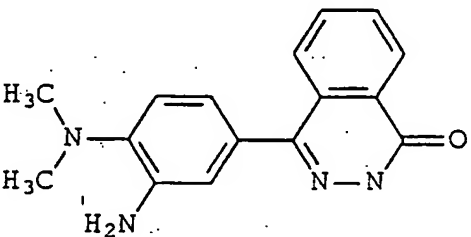
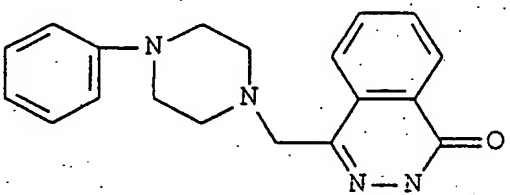
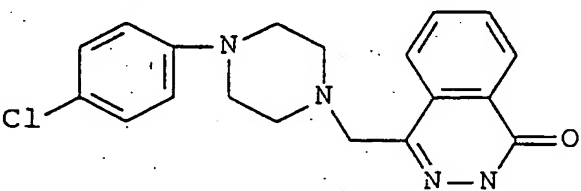
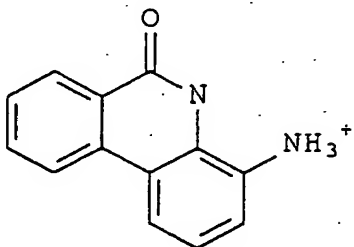
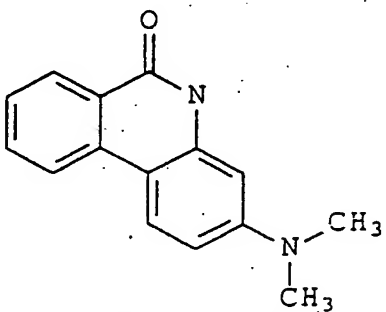
Compound	Approximate IC ₅₀ , _g
 <chem>Nc1ccc2c(c1)oc3ccccc3n2C(=O)N</chem>	0.046
 <chem>C1CCN(CC1)CCCCOc2ccc3c(c2)oc4ccccc4n3C(=O)N</chem>	1.9
 <chem>COC(=O)c1cnc2ccccc2c1C(=O)N</chem>	1.1
 <chem>Clc1ccc2c(c1)oc3ccccc3n2C(=O)N</chem>	1.6
 <chem>Clc1ccc(cc1)C(=N2C(=O)Nc3ccccc32)C(=O)N</chem>	0.36

Compound	Approximate IC ₅₀ 's
	1.9
	1.7
	0.40
	0.25
	0.60
	0.54

Compound	Approximate IC ₅₀ , _g
 <chem>Nc1ccc2c(c1)c(=O)[nH]c(=O)c2</chem>	0.09
 <chem>[K+].[O-]S(=O)(=O)c1ccc2c(c1)c(=O)[nH]c(=O)c2</chem>	4.0
 <chem>Clc1ccc2c(c1)c(=O)[nH]c(=O)c2</chem>	0.46
 <chem>OCCSc1ccc2c(c1)c(=O)[nH]c(=O)c2</chem>	2.8
 <chem>Oc1ccc2c(c1)c(=O)[nH]c(=O)c2</chem>	0.81

Compound	Approximate IC _{50's}
	5.1
	0.16
	0.10
	3.26
	0.30

5

Compound	Approximate IC _{50's}
	0.68
	0.22
	4.0
	1.3
	0.76
	0.31

Example 2: Neuroprotective Effect of DPQ on
 Focal Cerebral Ischemia in Rats

Focal cerebral ischemia was produced by cauterization of the right distal MCA (middle cerebral artery) with bilateral temporary common carotid artery occlusion in male Long-Evans rats for 90 minutes. All procedures performed on the animals were approved by the University Institutional Animal Care and Use Committee of the University of Pennsylvania. A total of 42 rats (weights: 230-340 g) obtained from Charles River were used in this study. The animals fasted overnight with free access to water prior to the surgical procedure.

Two hours prior to MCA occlusion, varying amounts (control, n=14; 5 mg/kg, n=7; 10 mg/kg, n=7; 20 mg/kg, n=7; and 40 mg/kg, n=7) of the compound, 3,4-dihydro-5-[4-(1-piperidinyl)-butoxy]-1(2H)-isoquinolinone ("DPQ") were dissolved in dimethyl sulfoxide (DMSO) using a sonicator. A volume of 1.28 ml/kg of the resulting solution was injected intraperitoneally into fourteen rats.

The rats were then anesthetized with halothane (4% for induction and 0.8%-1.2% for the surgical procedure) in a mixture of 70% nitrous oxide and 30% oxygen. The body temperature was monitored by a rectal probe and maintained at $37.5 \pm 0.5^{\circ}\text{C}$ with a heating blanket regulated by a homeothermic blanket control unit (Harvard Apparatus Limited, Kent, U.K.). A catheter (PE-50) was placed into the tail artery, and arterial pressure was continuously monitored and recorded on a Grass polygraph recorder (Model 7D, Grass Instruments, Quincy, Massachusetts). Samples for blood gas analysis (arterial pH, PaO_2 and PaCO_2) were also taken from the tail artery catheter and measured with a blood gas analyzer (ABL 30, Radiometer, Copenhagen, Denmark). Arterial blood samples were obtained 30 minutes after MCA occlusion.

The head of the animal was positioned in a stereotaxic frame, and a right parietal incision between the right lateral canthus and the external auditory meatus was made. Using a dental drill constantly cooled with saline, a 3 mm burr hole was prepared over the cortex supplied by the right MCA, 4 mm lateral to the sagittal suture and 5 mm caudal to the coronal suture. The dura mater and a thin inner bone layer were kept,

care being taken to position the probe over a tissue area devoid of large blood vessels. The flow probe (tip diameter of 1 mm, fiber separation of 0.25 mm) was lowered to the bottom of the cranial burr hole using a micromanipulator. The probe was
5 held stationary by a probe holder secured to the skull with dental cement. The microvascular blood flow in the right parietal cortex was continuously monitored with a laser Doppler flowmeter (FloLab, Moor, Devon, U.K., and Periflux 4001, Perimed, Stockholm, Sweden).

10 Focal cerebral ischemia was produced by cauterization of the distal portion of the right MCA with bilateral temporary common carotid artery (CCA) occlusion by the procedure of Chen et al., "A Model of Focal Ischemic Stroke in the Rat: Reproducible Extensive Cortical Infarction", *Stroke* 17:738-43
15 (1986) and/or Liu et al., "Polyethylene Glycol-conjugated Superoxide Dismutase and Catalase Reduce Ischemic Brain Injury", *Am. J. Physiol.* 256:H589-93 (1989), both of which are hereby incorporated by reference.

Specifically, bilateral CCA's were isolated, and loops
20 made from polyethylene (PE-10) catheter were carefully passed around the CCA's for later remote occlusion. The incision made previously for placement of the laser doppler probe was extended to allow observation of the rostral end of the zygomatic arch at the fusion point using a dental drill, and
25 the dura mater overlying the MCA was cut. The MCA distal to its crossing with the inferior cerebral vein was lifted by a fine stainless steel hook attached to a micromanipulator and, following bilateral CCA occlusion, the MCA was cauterized with an electrocoagulator. The burr hole was covered with a small
30 piece of Gelform, and the wound was sutured to maintain the brain temperature within the normal or near-normal range.

After 90 minutes of occlusion, the carotid loops were released, the tail arterial catheter was removed, and all of the wounds were sutured. Gentamicin sulfate (10 mg/ml) was
35 topically applied to the wounds to prevent infection. The anesthetic was discontinued, and the animal was returned to his cage after awakening. Water and food were allowed ad libitum.

Two hours after MCA occlusion, the animals were given the same doses of the PARP inhibitor as in the pre-treatment.

Twenty-four hours after MCA occlusion, the rats were sacrificed with an intraperitoneal injection of pentobarbital sodium (150 mg/kg). The brain was carefully removed from the skull and cooled in ice-cold artificial CSF for five minutes. The cooled
5 brain was then sectioned in the coronal plane at 2 mm intervals using a rodent brain matrix (RBM-4000C, ASI Instruments, Warren, Michigan). The brain slices were incubated in phosphate-buffered saline containing 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 37°C for ten minutes.
10 Color photographs were taken of the posterior surface of the stained slices and were used to determine the damaged area at each cross-sectional level using a computer-based image analyzer (NIH Image 1.59). To avoid artifacts due to edema, the damaged area was calculated by subtracting the area of the
15 normal tissue in the hemisphere ipsilateral to the stroke from the area of the hemisphere contralateral to the stroke, by the method of Swanson et al., "A Semiautomated Method for Measuring Brain Infarct Volume", *J. Cereb. Blood Flow Metabol.* 10:290-93 (1990), the disclosure of which is hereby incorporated by
20 reference. The total volume of infarction was calculated by summation of the damaged volume of the brain slices.

The cauterization of the distal portion of the right MCA with bilateral temporary CCA occlusion consistently produced a well-recognized cortical infarct in the right MCA territory of
25 each test animal. There was an apparent uniformity in the distribution of the damaged area as measured by TTC staining in each group, as shown in Figure 1.

In Figure 1, the distribution of the cross-sectional infarct area at representative levels along the rostrocaudal
30 axis was measured from the interaural line in non-treated animals and in animals treated with 10 mg/kg of 3,4-dihydro-5-[4-(1-piperidinyl)-butoxy]-1(2H)-isoquinolinone. The area of damage was expressed as mean \pm standard deviation. Significant differences between the 10 mg-treated group and the control
35 group were indicated ($p < 0.02$, $p < 0.01$, $p < 0.001$). The 5 mg/kg and 20 mg/kg curves fell approximately halfway between the control and the 10 mg/kg curves, whereas the 40 mg/kg curve was close to the control. The 5, 20 and 40 mg/kg curves were omitted for clarity.

PARP inhibition led to a significant decrease in the damaged volume in the 5 mg/kg-treated group ($106.7 \pm 23.2 \text{ mm}^3$, $p < 0.001$), the 10 mg/kg-treated group ($76.4 \pm 16.8 \text{ mm}^3$, $p < 0.001$), and the 20 mg/kg-treated group ($110.2 \pm 42.0 \text{ mm}^3$, $p < 0.01$), compared to the control group ($165.2 \pm 34.0 \text{ mm}^3$). The data are expressed as mean \pm standard deviation. The significance of differences between groups was determined using an analysis of variance (ANOVA) followed by Student's t-test for individual comparisons.

10 There was no significant difference between the control and the 40 mg/kg-treated group ($135.6 \pm 44.8 \text{ mm}^3$). However, there were significant differences between the 5 mg/kg-treated group and the 10 mg/kg-treated group ($p < 0.02$), and between the 10 mg/kg-treated group and the 40 mg/kg-treated group ($p < 0.01$),
15 as shown in Figure 2.

In Figure 2, the effect of intraperitoneal administration of 3,4-dihydro-5-[4-(1-piperidinyl)-butoxy]-1(2H)-isoquinolinone on the infarct volume was depicted graphically. The volumes of infarct were expressed as mean \pm standard
20 deviation. Significant differences between the treated groups and the control group were indicated ($p < 0.01$, $p < 0.001$). It is not clear why a high dose (40 mg/kg) of the PARP inhibitor, 3,4-dihydro-5-[4-(1-piperidinyl)-butoxy]-1(2H)-isoquinolinone, was less neuroprotective. The U-shaped dose-response curve may
25 suggest dual effects of the compound.

However, overall, the in vivo administration of the inhibitor led to a substantial reduction in infarct volume in the focal cerebral ischemia model in the rat. This result indicated that the activation of PARP plays an important role
30 in the pathogenesis of brain damage in cerebral ischemia.

The values of arterial blood gases (PaO_2 , PaCO_2 and pH) were within the physiological range in the control and treated groups with no significant differences in these parameters among the five groups, as shown below in Table 2. A "steady
35 state" MABP was taken following completion of the surgical preparation, just prior to occlusion; an "ischemia" MABP was taken as the average MABP during occlusion. See Table III below:

TABLE III

	PaO ₂ (mm Hg)	PaCO ₂ (mm Hg)	pH	MABP (mm Hg) Steady Ischemia State	
Control group (n=4)	125±21	38.6 ± 4.6	7.33 ± 0.05	79±14	91±13 ^{**}
5 mg/kg- treated group (n=7)	126±20	38.0 ± 2.8	7.36 ± 0.02	78± 5	91±12 ^{**}
10 mg/kg- treated group (n=7)	125±16	39.3 ± 5.2	7.34 ± 0.03	80± 9	90±14 [*]
20 mg/kg- treated group (n=7)	122±14	41.3 ± 2.8	7.35 ± 0.23	79±10	91±12 ^{**}
40 mg/kg- treated group (n=7)	137±17	39.5 ± 4.7	7.33 ± 0.24	78± 4	88±12 [*]

^{*} = Significantly different from the steady state value, p<0.05.

^{**} = Significantly different from the steady state value, p<0.01.

There were no significant differences in any physiological parameter, including mean arterial blood pressure (MABP), prior to MCA and CCA occlusion among the five groups. Although MABP was significantly elevated following occlusion in all five groups, there were no significant differences in MABP during the occlusion period among the groups.

Since the blood flow values obtained from the laser doppler were in arbitrary units, only percent changes from the baseline (prior to occlusion) were reported. Right MCA and bilateral CCA occlusion produced a significant decrease in relative blood flow in the right parietal cortex to 20.8 ± 7.7 % of the baseline in the control group (n=5), 18.7 ± 7.4 % in the 5 mg/kg-treated group (n=7), 21.4 ± 7.7 % in the 10 mg/kg-treated group (n=7) and 19.3 ± 11.2 % in the 40 mg/kg-treated group (n=7). There were no significant differences in the blood flow response to occlusion among the four groups. In addition, blood flow showed no significant changes throughout the entire occlusion period in any group.

Following release of the carotid occlusions, a good recovery of blood flow (sometimes hyperemia) was observed in the right MCA territory of all animals. Reperfusion of the

ischemic tissue resulted in the formation of NO and peroxynitrite, in addition to oxygen-derived free radicals. All of these radicals have been shown to cause DNA strand breaks and to activate PARP.

- 5 This example provided evidence that the related compounds of the present invention are effective in inhibiting PARP activity.

Example 3: Assay for Neuroprotective Effects on
Focal Cerebral Ischemia in Rats

- 10 Focal cerebral ischemia experiments are performed using male Wistar rats weighing 250 - 300 g, which are anesthetized with 4% halothane. Anesthesia is maintained with 1.0-1.5% halothane until the end of surgery. The animals are installed
15 in a warm environment to avoid a decrease in body temperature during surgery.

- An anterior midline cervical incision is made. The right common carotid artery (CCA) is exposed and isolated from the vagus nerve. A silk suture is placed and tied around the CCA
20 in proximity to the heart. The external carotid artery (ECA) is then exposed and ligated with a silk suture. A puncture is made in the CCA and a small catheter (PE 10, Ulrich & Co., St-Gallen, Switzerland) is gently advanced to the lumen of the internal carotid artery (ICA). The pterygopalatine artery is
25 not occluded. The catheter is tied in place with a silk suture. Then, a 4-0 nylon suture (Braun Medical, Crissier, Switzerland) is introduced into the catheter lumen and is pushed until the tip blocks the anterior cerebral artery. The length of catheter into the ICA is approximately 19 mm from the
30 origin of the ECA. The suture is maintained in this position by occlusion of the catheter with heat. One cm of catheter and nylon suture are left protruding so that the suture can be withdrawn to allow reperfusion. The skin incision is then closed with wound clips.

- 35 The animals are maintained in a warm environment during recovery from anesthesia. Two hours later, the animals are re-anesthetized, the clips are discarded, and the wound is re-opened. The catheter is cut, and the suture is pulled out. The catheter is then obturated again by heat, and wound clips
40 are placed on the wound. The animals are allowed to survive

for 24 hours with free access to food and water. The rats are then sacrificed with CO₂ and decapitated.

The brains are immediately removed, frozen on dry ice and stored at -80°C. The brains are then cut in 0.02 mm-thick sections in a cryocut at -19°C, selecting one of every 20 sections for further examination. The selected sections are stained with cresyl violet according to the Nissl procedure. Each stained section is examined under a light microscope, and the regional infarct area is determined according to the presence of cells with morphological changes.

Various doses of the compounds of the invention are tested in this model. The compounds are administered in either a single dose or a series of multiple doses, i.p. or i.v., at different times, both before or after the onset of ischemia. Compounds of the invention are found to provide protection from ischemia in the range of about 20 to 80%.

Example 4: Effects on Heart Ischemia/Reperfusion Injury in Rats

Female Sprague-Dawley rats, each weighing about 300-350 g are anesthetized with intraperitoneal ketamine at a dose of 150 mg/kg. The rats are endotracheally intubated and ventilated with oxygen-enriched room air using a Harvard rodent ventilator. Polyethylene catheters inserted into the carotid artery and the femoral vein are used for artery blood pressure monitoring and fluid administration respectively. Arterial pCO₂ is maintained between 35 and 45mm Hg by adjusting the respirator rate. The rat chests are opened by median sternotomy, the pericardium is incised, and the hearts are cradled with a latex membrane tent. Hemodynamic data are obtained at baseline after at least a 15-minute stabilization period following the end of the surgical operation. The LAD (left anterior descending) coronary artery is ligated for 40 minutes, and then re-perfused for 120 minutes. After 120 minutes' reperfusion, the LAD artery is re-occluded, and a 0.1 ml bolus of monastral blue dye is injected into the left atrium to determine the ischemic risk region.

The hearts are then arrested with potassium chloride and cut into five 2-3 mm thick transverse slices. Each slice is

weighed and incubated in a 1% solution of trimethyltetrazolium chloride to visualize the infarcted myocardium located within the risk region. Infarct size is calculated by summing the values for each left ventricular slice and is further expressed
5 as a fraction of the risk region of the left ventricle.

Various doses of the compounds of the invention are tested in this model. The compounds are given either in a single dose or a series of multiple doses, i.p. or i.v., at different times, both before or after the onset of ischemia. The
10 compounds of the invention are found to have ischemia/reperfusion injury protection in the range of 10 to 40 percent. Therefore, they protect against ischemia-induced degeneration of rat hippocampal neurons in vitro.

15 Example 5: Retinal Ischemia Protection

A patient just diagnosed with acute retinal ischemia is immediately administered parenterally, either by intermittent or continuous intravenous administration, a compound of formula I, either as a single dose or a series of divided doses of the
20 compound. After this initial treatment, and depending on the patient's presenting neurological symptoms, the patient optionally may receive the same or a different compound of the invention in the form of another parenteral dose. It is expected by the inventors that significant prevention of neural
25 tissue damage would ensue and that the patient's neurological symptoms would considerably lessen due to the administration of the compound, leaving fewer residual neurological effects post-stroke. In addition, it is expected that the re-occurrence of retinal ischemia would be prevented or reduced.

30

Example 6: Treatment of Retinal Ischemia

A patient has just been diagnosed with acute retinal ischemia. Immediately, a physician or a nurse parenterally administers a compound of formula I, either as a single dose or
35 as a series of divided doses. The patient also receives the same or a different PARP inhibitor by intermittent or continuous administration via implantation of a biocompatible, biodegradable polymeric matrix delivery system comprising a compound of formula I, or via a subdural pump inserted to

administer the compound directly to the infarct area of the brain. It is expected by the inventors that the patient would awaken from the coma more quickly than if the compound of the invention were not administered. The treatment is also
5 expected to reduce the severity of the patient's residual neurological symptoms. In addition, it is expected that re-occurrence of retinal ischemia would be reduced.

Example 7: Vascular Stroke Protection

10 A patient just diagnosed with acute vascular stroke is immediately administered parenterally, either by intermittent or continuous intravenous administration, a compound of formula IV, either as a single dose or a series of divided doses of the compound. After this initial treatment, and depending on the
15 patient's presenting neurological symptoms, the patient optionally may receive the same or a different compound of the invention in the form of another parenteral dose. It is expected by the inventors that significant prevention of neural tissue damage would ensue and that the patient's neurological
20 symptoms would considerably lessen due to the administration of the compound, leaving fewer residual neurological effects post-stroke. In addition, it is expected that the re-occurrence of vascular stroke would be prevented or reduced.

25 Example 8: Treatment of Vascular Stroke

A patient has just been diagnosed with acute multiple vascular strokes and is comatose. Immediately, a physician or a nurse parenterally administers a compound of formula IV, either as a single dose or as a series of divided doses. Due
30 to the comatose state of the patient, the patient also receives the same or a different PARP inhibitor by intermittent or continuous administration via implantation of a biocompatible, biodegradable polymeric matrix delivery system comprising a compound of formula IV, or via a subdural pump inserted to
35 administer the compound directly to the infarct area of the brain. It is expected by the inventors that the patient would awaken from the coma more quickly than if the compound of the invention were not administered. The treatment is also expected to reduce the severity of the patient's residual

neurological symptoms. In addition, it is expected that re-occurrence of vascular stroke would be reduced.

Example 9: Preventing Cardiac Reperfusion Injury

5 A patient is diagnosed with life-threatening cardiomyopathy and requires a heart transplant. Until a donor heart is found, the patient is maintained on Extra Corporeal Oxygenation Monitoring (ECMO).

10 A donor heart is then located, and the patient undergoes a surgical transplant procedure, during which the patient is placed on a heart-lung pump. The patient receives a compound of the invention intracardiac within a specified period of time prior to re-routing his or her circulation from the heart-lung pump to his or her new heart, thus preventing cardiac
15 reperfusion injury as the new heart begins to beat independently of the external heart-lung pump.

Example 10: Septic Shock Assay

Groups of 10 C57/BL male mice weighing 18 to 20 g were
20 administered a test compound, 1-carboxynaphthalene-1-carboxamide at the doses of 60, 20, 6 and 2 mg/kg, daily, by intraperitoneal (IP) injection for three consecutive days. Each animal was first challenged with lipopolysaccharide (LPS, from E. Coli, LD₅₀ of 20 mg/animal IV) plus galactosamine (20
25 mg/animal IV). The first dose of test compound in a suitable vehicle was given 30 minutes after challenge, and the second and third doses were given 24 hours later on day 2 and day 3 respectively, with only the surviving animals receiving the second or third dose of the test compound. Mortality was
30 recorded every 12 hours after challenge for the three-day testing period. 1-Carboxy-naphthalene-1-carboxamide provided a protection against mortality from septic shock of about 40%. Based on these results, other compounds of the invention are expected to provide a protection against mortality exceeding
35 about 35%.

Example 11: In vitro Radiosensitization

The human prostate cancer cell line, PC-3s, were plated in 6 well dishes and grown at monolayer cultures in RPMI1640

supplemented with 10% FCS. The cells are maintained at 37°C in 5% CO₂ and 95% air. The cells were exposed to a dose response (0.1 mM to 0.1 uM) of 3 different PARP-inhibitors of Formula I disclosed herein prior to irradiation at one sublethal dose level. For all treatment groups, the six well plates were exposed at room temperature in a Seifert 250kV/15mA irradiator with a 0.5 mm Cu/1 mm. Cell viability was examined by exclusion of 0.4% trypan blue. Dye exclusion was assessed visually by microscopy and viable cell number was calculated by subtracting the number of cells from the viable cell number and dividing by the total number of cells. Cell proliferation rates were calculated by the amount of ³H-thymidine incorporation post-irradiation. The PARP inhibitors show radiosensitization of the cells.

15

Example 12 In vivo Radiosensitization

Before undergoing radiation therapy to treat cancer, a patient is administered an effective amount of a compound or a pharmaceutical composition of the present invention. The compound or pharmaceutical composition acts as a radiosensitizer and making the tumor more susceptible to radiation therapy.

20

Example 13 Measuring Altered Gene Expression in mRNA Senescent Cells

25

Human fibroblast BJ cells, at Population Doubling (PDL) 94, are plated in regular growth medium and then changed to low serum medium to reflect physiological conditions described in Linskens, et al., *Nucleic Acids Res.* 23:16:3244-3251 (1995). A medium of DMEM/199 supplemented with 0.5% bovine calf serum is used. The cells are treated daily for 13 days with the PARP inhibitor of Formula I as disclosed herein. The control cells are treated with and without the solvent used to administer the PARP inhibitor. The untreated old and young control cells are tested for comparison. RNA is prepared from the treated and control cells according to the techniques described in PCT Publication No. 96/13610 and Northern blotting is conducted. Probes specific for senescence-related genes are analyzed, and treated and control cells compared. In analyzing the results,

30

35

the lowest level of gene expression is arbitrarily set at 1 to provide a basis for comparison. Three genes particularly relevant to age-related changes in the skin are collagen, collagenase and elastin. West, *Arch. Derm.* 130:87-95 (1994).

- 5 Elastin expression of the cells treated with the PARP inhibitor of Formula I is significantly increased in comparison with the control cells. Elastin expression is significantly higher in young cells compared to senescent cells, and thus treatment with the PARP inhibitor of Formula I causes elastin expression
10 levels in senescent cells to change to levels similar to those found in much younger cells. Similarly, a beneficial effect is seen in collagenase and collagen expression with treatment with the PARP inhibitors of Formula I.

15 Example 14 Measuring Altered Gene Expression
 Protein in Senescent Cells

- Approximately 105 BJ cells, at PDL 95-100 are plated and grown in 15 cm dishes. The growth medium is DMEM/199 supplemented with 10% bovine calf serum. The cells are treated
20 daily for 24 hours with the PARP inhibitors of Formula I (100 ug/ 1 mL of medium). The cells are washed with phosphate buffered solution (PBS), then permeablized with 4% paraformaldehyde for 5 minutes, then washed with PBS, and treated with 100% cold methanol for 10 minutes. The methanol
25 is removed and the cells are washed with PBS, and then treated with 10% serum to block nonspecific antibody binding. About 1 mL of the appropriate commercially available antibody solutions (1:500 dilution. Vector) is added to the cells and the mixture incubated for 1 hour. The cells are rinsed and washed three
30 times with PBS. A secondary antibody, goat anti-mouse IgG (1 mL) with a biotin tag is added along with 1 mL of a solution containing streptavidin conjugated to alkaline phosphatase and 1 mL of NBT reagent (Vector). The cells are washed and changes in gene expression are noted colorimetrically. Four
35 senescence-specific genes -- collagen I, collagen III, collagenase, and interferon gamma -- in senescent cells treated with the PARP inhibitor of Formula I are monitored and the results show a decrease in interferon gamma expression with no observable change in the expression levels of the other three

gens, demonstrating that the PARP inhibitors of Formula I can alter senescence-specific gene expression.

5 Example 15 Extending or Increasing Proliferative
 Capacity and Lifespan of Cells

To demonstrate the effectiveness of the present method for extending the proliferative capacity and lifespan of cells, human fibroblast cells lines (either W138 at Population Doubling (PDL) 23 or BJ cells at PDL 71) are thawed and plated
10 on T75 flasks and allowed to grow in normal medium (DMEM/M199 plus 10% bovine calf serum) for about a week, at which time the cells are confluent, and the cultures are therefor ready to be subdivided. At the time of subdivision, the media is aspirated, and the cells rinsed with phosphate buffer saline
15 (PBS) and then trypsinized. The cells are counted with a Coulter counter and plated at a density of 10^5 cells per cm^2 in 6-well tissue culture plates in DMEM/199 medium supplemented with 10% bovine calf serum and varying amounts (0.10uM, and 1mM: from a 100X stock solution in DMEM/M199 medium) of a PARP
20 inhibitor of Formula I as disclosed herein. This process is repeated every 7 days until the cell appear to stop dividing. The untreated (control) cells reach senescence and stop dividing after about 40 days in culture. Treatment of cells with 10 uM 3-AB appears to have little or no effect in contrast
25 to treatment with 100 uM 3-AB which appears lengthen the lifespan of the cells and treatment with 1 mM 3-AB which dramatically increases the lifespan and proliferative capacity of the cells. The cells treated with 1 mM 3-AB will still divide after 60 days in culture.

30

Example 16: Neuroprotective Effects of Formula I on
 Chronic Constriction Injury (CCI) in Rats

Adult male Sprague-Dawley rats, 300-350 g, are anesthetized with intraperitoneal 50 mg/kg sodium
35 pentobarbital. Nerve ligation is performed by exposing one side of the rat's sciatic nerves and dissecting a 5-7 mm-long nerve segment and closing with four loose ligatures at a 1.0-1.5-mm, followed by implanting of an intrathecal catheter and inserting of a gentamicin sulfate-flushed polyethylene (PE-10)
40 tube into the subarachnoid space through an incision at the

cisterna magna. The caudal end of the catheter is gently threaded to the lumbar enlargement and the rostral end is secured with dental cement to a screw embedded in the skull and the skin wound is closed with wound clips.

- 5 Thermal hyperalgesia to radiant heat is assessed by using a paw-withdrawal test. The rat is placed in a plastic cylinder on a 3-mm thick glass plate with a radiant heat source from a projection bulb placed directly under the plantar surface of the rat's hindpaw. The paw-withdrawal latency is defined as
10 the time elapsed from the onset of radiant heat stimulation to withdrawal of the rat's hindpaw.

Mechanical hyperalgesia is assessed by placing the rat in a cage with a bottom made of perforated metal sheet with many small square holes. Duration of paw-withdrawal is recorded
15 after pricking the mid-plantar surface of the rat's hindpaw with the tip of a safety pin inserted through the cage bottom.

Mechano-allodynia is assessed by placing a rat in a cage similar to the previous test, and applying von Frey filaments in ascending order of bending force ranging from 0.07 to 76 g
20 to the mid-plantar surface of the rat's hindpaw. A von Frey filament is applied perpendicular to the skin and depressed slowly until it bends. A threshold force of response is defined as the first filament in the series to evoke at least one clear paw-withdrawal out of five applications.

- 25 Dark neurons are observed bilaterally within the spinal cord dorsal horn, particularly in laminae I-II, of rats 8 days after unilateral sciatic nerve ligation as compared with sham operated rats. Various doses of differing compounds of Formula I are tested in this model and show that the Formula I
30 compounds reduce both incidence of dark neurons and neuropathic pain behavior in CCI rats.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are
35 not to be regarded as a departure from the spirit and scope of the invention, and all such modifications are intended to be included within the scope of the following claims.

We claim:

1. A compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

5

2. The compound of claim 1, wherein the neuronal activity is selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration, and treatment of a
10 neurological disorder.

3. The compound of claim 2, wherein the neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state,
15 traumatic brain injury, physical damage to the spinal cord, stroke, demyelinating disease and neurological disorder relating to neurodegeneration.

4. The compound of claim 3, wherein the neurological
20 disorder is stroke.

5. The compound of claim 3, wherein the neurological disorder relating to neurodegeneration is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease,
25 Huntington's Disease, and amyotrophic lateral sclerosis.

6. The compound of claim 1, which forms at least one hydrogen bond with an amino acid in PARP.

30 7. The compound of claim 6, wherein the amino acid is Gly.

8. The compound of claim 7, wherein said compound contains an NH group which forms a hydrogen bond with the O
35 atom of Gly.

9. The compound of claim 8, wherein the length of said bond is about 1-5 Å.

10. The compound of claim 9, wherein the length of said bond is about 2-4 Å.

11. The compound of claim 6, wherein the amino acid is Ser.

12. The compound of claim 11, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

13. The compound of claim 12, wherein the length of said bond is about 1-5 Å.

14. The compound of claim 13, wherein the length of said bond is about 2-4 Å.

15. The compound of claim 1, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 100 μM or lower.

16. The compound of claim 15, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 25 μM or lower.

17. A compound which inhibits PARP activity and treats or prevents tissue damage resulting from cell death or damage due to necrosis or apoptosis.

18. The compound of claim 17 further comprising treating or preventing diseases or conditions selected from the group consisting of tissue damage resulting from cell damage or death due to necrosis or apoptosis, neuronal mediated tissue damage or diseases, neural tissue damage resulting from ischemia and reperfusion injury, neurological disorders and neurodegenerative diseases, vascular stroke, cardiovascular disorders, age-related macular degeneration, AIDS and other immune senescence diseases, arthritis, atherosclerosis, cachexia, cancer, degenerative diseases of skeletal muscle involving replicative senescence, diabetes, head trauma, immune senescence, inflammatory bowel disorders, muscular dystrophy, osteoarthritis, osteoporosis, chronic pain, acute pain,

neuropathic pain, nervous insult, peripheral nerve injury, renal failure, retinal ischemia, septic shock, and skin aging, diseases or disorders relating to lifespan or proliferative capacity of cells, and diseases or disease conditions induced
5 or exacerbated by cellular senescence.

19. A pharmaceutical composition comprising:

- (i) an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA
10 toxicity; and
- (ii) a pharmaceutically acceptable carrier.

20. The pharmaceutical composition of claim 19, wherein the neuronal activity is selected from the group consisting of
15 stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration, and treatment of a neurological disorder.

21. The pharmaceutical composition of claim 20, wherein
20 the neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke, demyelinating disease and neurological disorder relating to neurodegeneration.

22. The pharmaceutical composition of claim 21, wherein the neurological disorder is stroke.

23. The pharmaceutical composition of claim 21, wherein
30 the neurological disorder relating to neurodegeneration is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis.

24. The pharmaceutical composition of claim 19, wherein
35 said compound forms at least one hydrogen bond with an amino acid in PARP.

25. The pharmaceutical composition of claim 24, wherein

the amino acid is Gly.

26. The pharmaceutical composition of claim 25, wherein said compound contains an NH group which forms a hydrogen bond with the O atom of Gly.

27. The pharmaceutical composition of claim 26, wherein the length of said bond is about 1-5 Å.

10 28. The pharmaceutical composition of claim 27, wherein the length of said bond is about 2-4 Å.

29. The pharmaceutical composition of claim 24, wherein the amino acid is Ser.

15

30. The pharmaceutical composition of claim 29, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

20 31. The pharmaceutical composition of claim 30, wherein the length of said bond is about 1-5 Å.

32. The pharmaceutical composition of claim 31, wherein the length of said bond is about 2-4 Å.

25

33. The pharmaceutical composition of claim 19, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 100 μM or lower.

30 34. The pharmaceutical composition of claim 33, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 25 μM or lower.

35 35. The pharmaceutical composition of claim 19, wherein said compound is present in an amount that is effective for treating a cardiovascular disorder in an animal.

36. The pharmaceutical composition of claim 35, wherein the cardiovascular disorder is selected from the group

consisting of coronary artery disease, angina pectoris, myocardial infarction, cardiac arrest, cardiogenic shock, and cardiovascular tissue damage.

5 37. The pharmaceutical composition of claim 19, wherein said compound is present in an amount that is effective for treating organ damage due to transplantation.

10 38. The pharmaceutical composition of claim 19, wherein said compound is present in an amount that is effective for treating an inflammatory bowel disorder in an animal.

15 39. The pharmaceutical composition of claim 38, wherein the inflammatory bowel disorder is Crohn's Disease.

 40. The pharmaceutical composition of claim 38, wherein the inflammatory bowel disorder is colitis.

20 41. The pharmaceutical composition of claim 19, wherein said compound is present in an amount that is effective for treating arthritis in an animal.

25 42. The pharmaceutical composition of claim 19, wherein said compound is present in an amount that is effective for treating diabetes in an animal.

30 43. The pharmaceutical composition of claim 19, wherein said compound is present in an amount that is effective for treating septic shock in an animal.

 44. The pharmaceutical composition of claim 43, wherein the septic shock is endotoxic shock.

35 45. The pharmaceutical composition of claim 43, wherein the septic shock is acute tubular necrosis.

 46. The pharmaceutical composition of claim 19, wherein said compound is present in an amount that is effective for treating cancer in an animal.

47. The pharmaceutical composition of claim 46, wherein the cancer is selected from the group consisting of ACTH-producing tumors, acute lymphocytic leukemia, acute nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma, ovarian cancer, ovarian (germ cell) cancer, pancreatic cancer, penile cancer, prostate cancer, retinoblastoma, skin cancer, soft tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva, and Wilm's tumor.

20

48. A pharmaceutical composition comprising:

- (a) a compound which inhibits PARP activity and treats or prevents tissue damage resulting from cell death or damage due to necrosis or apoptosis; and
- (b) a pharmaceutically acceptable carrier.

25

49. The compound of claim 48 further comprising treating or preventing diseases or conditions selected from the group consisting of tissue damage resulting from cell damage or death due to necrosis or apoptosis, neuronal mediated tissue damage or diseases, neural tissue damage resulting from ischemia and reperfusion injury, neurological disorders and neurodegenerative diseases, vascular stroke, cardiovascular disorders, age-related macular degeneration, AIDS and other immune senescence diseases, arthritis, atherosclerosis, cachexia, cancer, degenerative diseases of skeletal muscle involving replicative senescence, diabetes, head trauma, immune senescence, inflammatory bowel disorders, muscular dystrophy, osteoarthritis, osteoporosis, chronic pain, acute pain,

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neuropathic pain, nervous insult, peripheral nerve injury, renal failure, retinal ischemia, septic shock, and skin aging, diseases or disorders relating to lifespan or proliferative capacity of cells, and diseases or disease conditions induced
5 or exacerbated by cellular senescence.

50. A method of effecting a neuronal activity not mediated by NMDA toxicity in an animal comprising administering an effective amount of a compound which inhibits PARP activity.
10

51. The method of claim 50, wherein the neuronal activity is selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration, and treatment of a neurological disorder.
15

52. The method of claim 51, wherein the neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord,
20 stroke, demyelinating disease and neurological disorder relating to neurodegeneration.

53. The method of claim 52, wherein the neurological disorder is stroke.
25

54. The method of claim 52, wherein the neurological disorder relating to neurodegeneration is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis.
30

55. The method of claim 50, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

56. The method of claim of claim 55, wherein the amino
35 acid is Gly.

57. The method of claim 56, wherein said compound contains an NH group which forms a hydrogen bond with the O atom of Gly.

58. The method of claim 57, wherein the length of said bond is about 1-5 Å.

59. The method of claim 58, wherein the length of said bond is about 2-4 Å.

60. The method of claim 55, wherein the amino acid is Ser.

10 61. The method of claim 60, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

62. The method of claim 61, wherein the length of said bond is about 1-5 Å.

63. The method of claim 62, wherein the length of said bond is about 2-4 Å.

20 64. The method of claim 50, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 100 μ M or lower.

65. The method of claim 64, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 25 μ M or lower.

25

66. A method of treating a cardiovascular disorder in an animal, comprising administering to said animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

30

67. The method of claim 66, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

68. The method of claim 67, wherein the amino acid is Gly.

35

69. The method of claim 68, wherein said compound contains an NH group which forms a hydrogen bond with the O atom of Gly.

70. The method of claim 69, wherein the length of said bond is about 1-5 Å.

71. The method of claim 70, wherein the length of said 5 bond is about 2-4 Å.

72. The method of claim 67, wherein the amino acid is Ser.

10 73. The method of claim 72, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

74. The method of claim 73, wherein the length of said 15 bond is about 1-5 Å.

75. The method of claim 74, wherein the length of said bond is about 2-4 Å.

20 76. The method of claim 66, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 100 uM or lower.

77. The method of claim 76, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 25 uM or lower.

25 78. The method of claim 66, wherein the cardiovascular disorder is selected from the group consisting of coronary artery disease, angina pectoris, myocardial infarction, cardiac arrest, cardiogenic shock, and cardiovascular tissue damage.

30 79. A method of treating organ damage due to transplantation in an animal, comprising administering to said animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA 35 toxicity.

80. The method of claim 79, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

81. The method of claim 80, wherein the amino acid is Gly.

82. The method of claim 81, wherein said compound
5 contains an NH group which forms a hydrogen bond with the O atom of Gly.

83. The method of claim 82, wherein the length of said bond is about 1-5 Å.

10

84. The method of claim 83, wherein the length of said bond is about 2-4 Å.

85. The method of claim 84, wherein the amino acid is
15 Ser.

86. The method of claim 85, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

20

87. The method of claim 86, wherein the length of said bond is about 1-5 Å.

88. The method of claim 87, wherein the length of said
25 bond is about 2-4 Å.

89. The method of claim 79, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 100 uM or lower.

30 90. The method of claim 89, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 25 uM or lower.

91. A method of treating an inflammatory bowel disorder in an animal, comprising administering to said animal an
35 effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

92. The method of claim 91, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

93. The method of claim 92, wherein the amino acid is Gly.

94. The method of claim 93, wherein said compound
5 contains an NH group which forms a hydrogen bond with the O atom of Gly.

95. The method of claim 94, wherein the length of said bond is about 1-5 Å.

10

96. The method of claim 95, wherein the length of said bond is about 2-4 Å.

97. The method of claim 92, wherein the amino acid is
15 Ser.

98. The method of claim 97, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

20

99. The method of claim 98, wherein the length of said bond is about 1-5 Å.

100. The method of claim 99, wherein the length of said
25 bond is about 2-4 Å.

101. The method of claim 91, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 100 uM or lower.

30 102. The method of claim 101, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 25 uM or lower.

103. The method of claim 91, wherein the inflammatory bowel disorder is Crohn's Disease.

35

104. The method of claim 91, wherein the inflammatory bowel disorder is colitis.

105. A method of treating arthritis in an animal,

comprising administering to said animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity

5 106. The method of claim 105, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

107. The method of claim 106, wherein the amino acid is Gly.

10

108. The method of claim 107, wherein said compound contains an NH group which forms a hydrogen bond with the O atom of Gly.

15

109. The method of claim 108, wherein the length of said bond is about 1-5 Å.

110. The method of claim 109, wherein the length of said bond is about 2-4 Å.

20

111. The method of claim 106, wherein the amino acid is Ser.

112. The method of claim 111, wherein said compound
25 contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

113. The method of claim 112, wherein the length of said bond is about 1-5 Å.

30

114. The method of claim 113, wherein the length of said bond is about 2-4 Å.

115. The method of claim 105, wherein said compound has an
35 IC₅₀ for inhibiting PARP in vitro of 100 µM or lower.

116. The method of claim 115, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 25 µM or lower.

117. A method of treating diabetes in an animal, comprising administering to said animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

5

118. The method of claim 117, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

119. The method of claim 118, wherein the amino acid is
10 Gly.

120. The method of claim 119, wherein said compound contains an NH group which forms a hydrogen bond with the O atom of Gly.

15

121. The method of claim 120, wherein the length of said bond is about 1-5 Å.

122. The method of claim 121, wherein the length of said
20 bond is about 2-4 Å.

123. The method of claim 118, wherein the amino acid is Ser.

25 124. The method of claim 123, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

125. The method of claim 124, wherein the length of said
30 bond is about 1-5 Å.

126. The method of claim 125, wherein the length of said bond is about 2-4 Å.

35 127. The method of claim 117, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 100 uM or lower.

128. The method of claim 127, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 25 uM or lower.

129. A method of treating septic shock in an animal, comprising administering to said animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

5

130. The method of claim 129, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

131. The method of claim 130, wherein the amino acid is
10 Gly.

132. The method of claim 131, wherein said compound contains an NH group which forms a hydrogen bond with the O atom of Gly.

15

133. The method of claim 132, wherein the length of said bond is about 1-5 Å.

134. The method of claim 133, wherein the length of said
20 bond is about 2-4 Å.

135. The method of claim 130, wherein the amino acid is Ser.

25 136. The method of claim 135, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

137. The method of claim 136, wherein the length of said
30 bond is about 1-5 Å.

138. The method of claim 137, wherein the length of said bond is about 2-4 Å.

35 139. The method of claim 129, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 100 μM or lower.

140. The method of claim 139, wherein said compound has an IC₅₀ for inhibiting PARP in vitro of 25 μM or lower.

141. The method of claim 129, wherein the septic shock is endotoxic shock.

142. The method of claim 129, wherein the septic shock is
5 acute tubular necrosis.

143. A method of treating cancer in an animal, comprising administering to said animal an effective amount of a compound which inhibits PARP activity and effects a neuronal activity
10 not mediated by NMDA toxicity.

144. The method of claim 143, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

15 145. The method of claim 144, wherein the amino acid is Gly.

146. The method of claim 145, wherein said compound contains an NH group which forms a hydrogen bond with the O
20 atom of Gly.

147. The method of claim 146, wherein the length of said bond is about 1-5 Å.

25 148. The method of claim 147, wherein the length of said bond is about 2-4 Å.

149. The method of claim 144, wherein the amino acid is Ser.

30 150. The method of claim 150, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

35 151. The method of claim 151, wherein the length of said bond is about 1-5 Å.

152. The method of claim 152, wherein the length of said bond is about 2-4 Å.

153. The method of claim 143, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 100 μ M or lower.

154. The method of claim 153, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 25 μ M or lower.

155. The method of claim 155, wherein the cancer is selected from the group consisting of ACTH-producing tumors, acute lymphocytic leukemia, acute nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma, ovarian cancer, ovarian (germ cell) cancer, pancreatic cancer, penile cancer, prostate cancer, retinoblastoma, skin cancer, soft tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva, and Wilm's tumor.

156. A method of inhibiting PARP activity comprising administering an effective amount of a compound which inhibits PARP activity and effects a neuronal activity not mediated by NMDA toxicity.

157. The method of claim 156, wherein said compound forms at least one hydrogen bond with an amino acid in PARP.

158. The method of claim 157, wherein the amino acid is Gly.

159. The method of claim 158, wherein said compound contains an NH group which forms a hydrogen bond with the O atom of Gly.

160. The method of claim 159, wherein the length of said bond is about 1-5 Å.

161. The method of claim 160, wherein the length of said bond is about 2-4 Å.

162. The method of claim 157, wherein the amino acid is Ser.

10 163. The method of claim 162, wherein said compound contains an O, S or N atom which forms a hydrogen bond with the H atom of Ser.

164. The method of claim 163, wherein the length of said bond is about 1-5 Å.

165. The method of claim 164, wherein the length of said bond is about 2-4 Å.

20 166. The method of claim 156, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 100 μM or lower.

167. The method of claim 166, wherein said compound has an IC_{50} for inhibiting PARP in vitro of 25 μM or lower.

25 168. A method of inhibiting PARP activity comprising administering an effective amount of a compound which inhibits PARP activity and treats or prevents tissue damage resulting from cell death or damage due to necrosis or apoptosis.

30 169. A method of extending or increasing lifespan and proliferative capacity of cells comprising administering an effective amount of a compound which inhibits PARP activity.

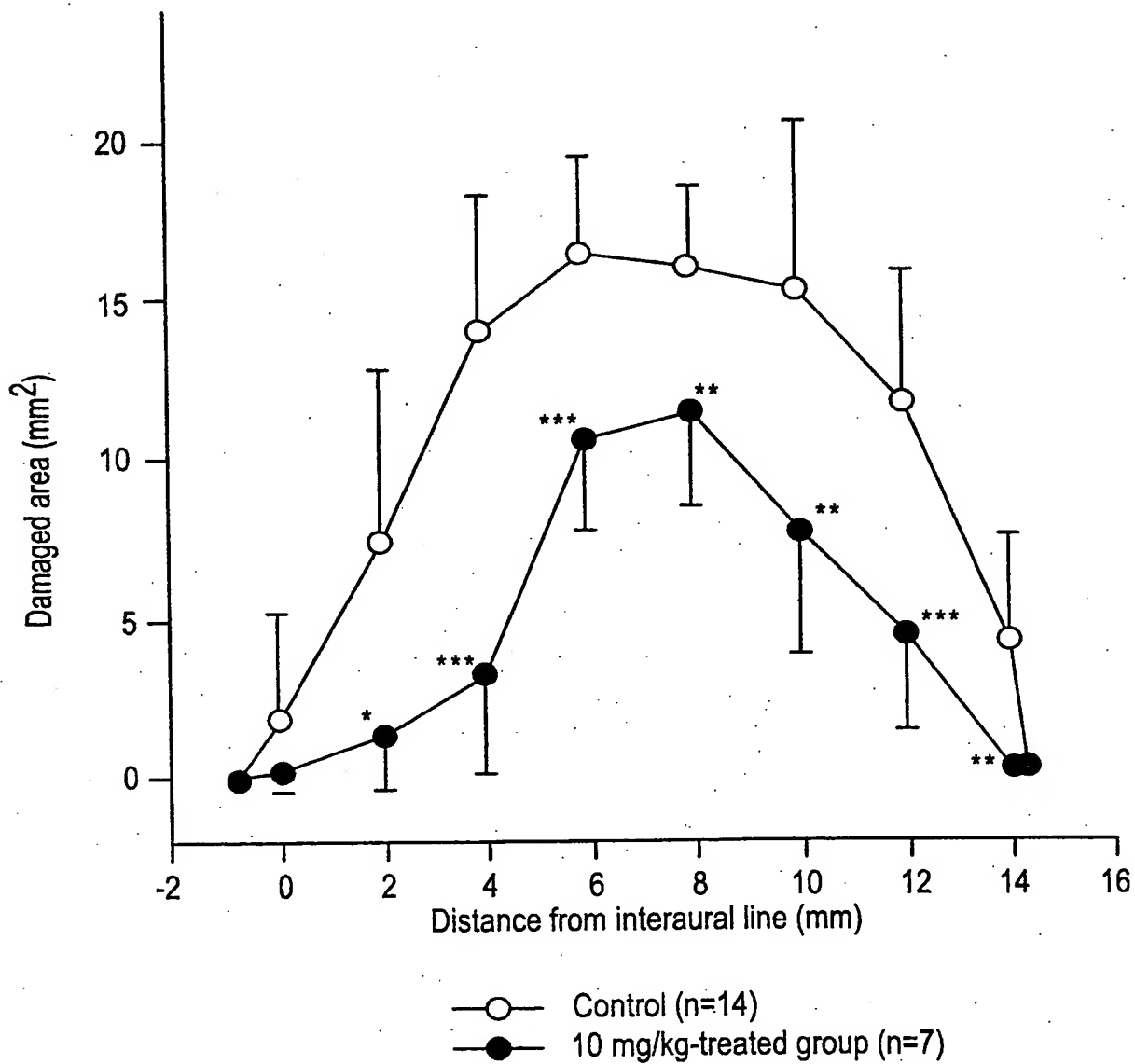
35 170. A method of altering gene expression of senescent cells comprising administering an effective amount of a compound which inhibits PARP activity.

171. The compounds, pharmaceutical compositions, methods

and processes described herein.

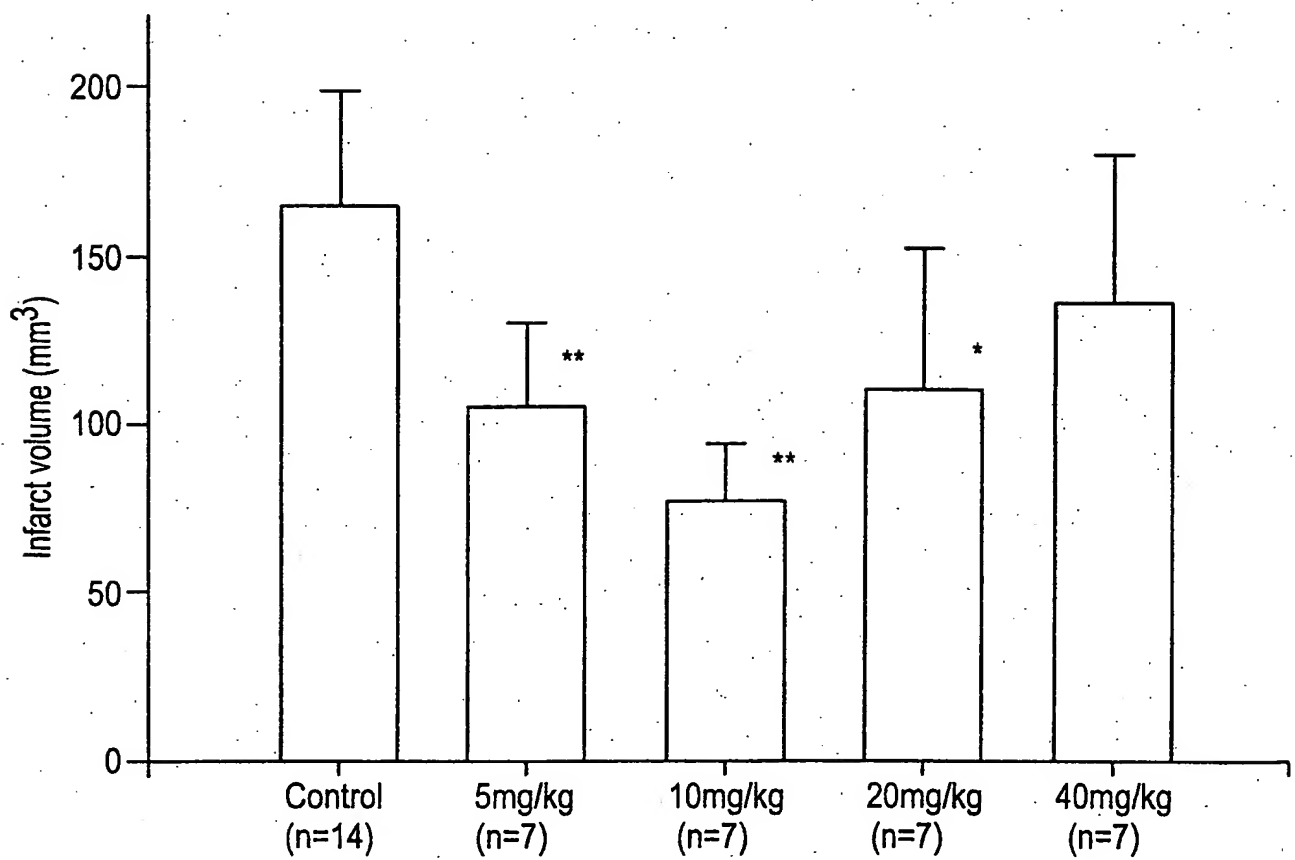
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FIG. 1



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FIG. 2



PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a) and Rule 39)

REC'D 23 DEC 1998
WIPO PCT

Applicant's or agent's file reference 23532X-PCT	IMPORTANT DECLARATION	Date of mailing (day/month/year) 21/12/1998
International application No. PCT/US 98/ 18185	International filing date (day/month/year) 02/09/1998	(Earliest) Priority date (day/month/year) 03/09/1997
International Patent Classification (IPC) or both national classification and IPC		C07G17/00 A61K31/00
Applicant GUILFORD PHARMACEUTICALS INC.		

This International Searching Authority hereby declares, according to Article 17(2)(a), that no international search report will be established on the international application for the reasons indicated below

1. ☐ The subject matter of the international application relates to:
 - a. ☐ scientific theories.
 - b. ☐ mathematical theories
 - c. ☐ plant varieties.
 - d. ☐ animal varieties.
 - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
 - f. ☐ schemes, rules or methods of doing business.
 - g. ☐ schemes, rules or methods of performing purely mental acts.
 - h. ☐ schemes, rules or methods of playing games.
 - i. ☐ methods for treatment of the human body by surgery or therapy.
 - j. ☐ methods for treatment of the animal body by surgery or therapy.
 - k. ☐ diagnostic methods practised on the human or animal body.
 - l. ☐ mere presentations of information.
 - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.
2. ☒ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

☐ the description
☒ the claims
☐ the drawings
3. ☐ The failure of the nucleotide and/or amino acid sequence listing to comply with the prescribed requirements prevents a meaningful search from being carried out:

☐ it does not comply with the prescribed standard
☐ it is not in the prescribed machine readable form
4. Further comments:

SEE ANNEX

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Ralf Ockers
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NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether:

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

The subject matter of the present claims relates to chemical compounds functionally characterised by specific biological effects only, to pharmaceutical compositions containing and methods using said compounds.

The claims 1-49, and 171, relating to compounds (claims 1-18, 49, 171) and pharmaceutical compositions (19-48, 171) are deprived of any technical feature that could serve to formulate the subject of a meaningful search as they lack any definition which would allow to construe a structural chemical representation (even not a partial one) of the compounds fulfilling these specific requirements. If a physical entity (in the present case a chemical compound), which represents a special technical feature of a claim, is merely defined by means of a (an enumeration of) functional feature(s) (in the present case a biological activity), such a claim lacks clarity.

It is therefore concluded that the present claims do not fulfill the requirement of clarity of Article 6 PCT to such an extent that a meaningful search is not possible.

The claims 50-171 relate to method for treatment of the human body by therapy, subject matter for which the EPO acting as ISA is not required to search an international application (Rule 39.1 (iv) PCT). Moreover the definition of the compounds and compositions defined in these claims present the same deficiencies as those indicated for the compounds and compositions of claims 1-49 and as a consequence it is not possible either to execute a search, as far as the claims 50-171 are concerned, oriented towards the technical effect of the compounds and compositions mentioned in the claims 50-171.

As it can not be foreseen to which subject matter present in the description the claims might reasonably be directed after amendments, the ISA is of the opinion that in accordance with Article 17.2) a) (i) and (ii) PCT no meaningful search can be carried out for the subject matter of the present claims, or for the subject matter present in the application to which amended claims might be drawn; consequently no international search report will be established.

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